

Application of Scanning Electron Microscopy Unravelling the Seed Coat Micro Morphology and FESEM-EDAX Characterization of Selected Species of the Genus *Rotala* L.(Lythraceae) in India

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ABSTRACT

The taxonomic importance of seed coat micromorphological and macromorphological traits among 10 species of the genera *Rotala*, was investigated by using Scanning Electron Microscope (SEM). The study aimed to identify unique ultra-structural characteristics of seed coat that would be useful for identification, helping to accurately determine and clarify their taxonomic position. This study explored the ultrastructure and micromorphological diversity among the examined genera, Morphological characters, including colour, texture, shape, hilum position, anticlinal and periclinal shape of seed coat were investigated. The highest seed length/width ratio was observed in *R. occultiflora*, 2.576 mm, whereas the lowest seed length/width ratio was found in *R. malabarica*, 1.154mm. The most prominent seed coat sculpture is reticulate type. Hilum tip is acute in all studied genera except *R. kasaragodensis* where it is round in shape. These characteristics proved valuable for distinguishing species within the studied genera and provided strong taxonomic insights. Our findings highlight that SEM-based examination of seed coat micromorphology can serve as a reliable tool for identifying plant taxa at both genus and species levels, offering potential for new taxonomic discoveries.

Keywords: Identification; India; Lythraceae; Morphology; *Rotala*; Scanning Electron Microscope; Seed Coat

INTRODUCTION

The genus *Rotala* L. (Lythraceae) is distributed tropically and subtropically. It is represented globally by 44 species (Mabberley, 2005), with the highest distribution in tropical Asia (Cook, 1979). After Cook's revision, it is represented, as per the revised estimate, in India by 29 species, of which 24 are from Peninsular India (Joseph & Sivar 1988, 1989; Pradcep & al, 1990; Mathew & Lekshminarasimhan, 1990; Yadav & al. 2010; Prasad & al. 2012; Gaikwad & al. 2013; Prasad & Raveendran 2013 a, b; Sunil & al. 2013; Anto & al. 2014; Ratheesh Narayanan & al. 2014; Lemiya & Pradeep 2015; Prasanth & Sardesai 2022). Species of the genus *Rotala* are grouped under two categories, based on the nature of their habitat, viz., obligate aquatics, which grow in shallow water, and semi aquatics or terrestrials, which thrive in marshy lands. Most of the aquatic species are characterized by what is called 'Hippuris syndrome' (Cook 1978): erect, unbranched stems with simple, elongate leaves borne in symmetrical whorls, "heterophylly manifesting itself as variation in some leaves in each whorl and individual leaf shape and size." Among the Indian species, this syndrome is displayed by *R. verticillaris* L., *R. mexicana* (only where it grows as an emergent aquatic), *R. cookie* K. T. Joseph and *R. vasodevanii* K. T. Joseph & Sivar. Most other species have decussate leaves, while *R. floribunda* (Wight) Koehne has alternate ones, and *R. occultiflora* has leaves disposed in whorls of 3. Leaf and flower arrangements have been taken as an important taxonomic character

in this genus by many authors. Koehne (1903) divided the genus into two sections based on these characters: Sect. Hippiuridum with whorled/alternate leaves and flowers, and Sect. Enantiorotala with decussate leaves and flowers, but species like *R. mexicana* and *R. myriophyllodes* Welw. ex Hieron display that both their characteristics depending upon whether they are growing as aquatics or terrestrials. Consequently, Cook (1978, 1979) considered these characters to be direct responses to the aquatic environment and that these 'Hippurismimics' do not constitute a single phyletic group. Cook (1979) has also found that the genus is very uniform and does not yield a satisfactory subgeneric classification into natural subgeneric groups. Seed coat characteristics have been successfully employed to solve taxonomic problems and elucidate phylogenetic relationships. Seed micromorphological studies using FESEM and EDAX have proven to be powerful tools for investigating features of taxonomic significance. Seed micromorphological characters are conserved and stable and thus used to solve many taxonomic ambiguities. Anatomical and seed micromorphological features can be used in taxonomic analysis for the identification of plants and establishing genetic relationships to solve taxonomic disputes.

The genus *Rotala* includes annual herbaceous plants, that inhabit permanent and seasonal lateritic pools, depressions of lateritic rocks with rich humus deposits, paddy fields, and marshy areas. Leaves are alternate, opposite, or whorled. It may be petiolate or sessile, lanceolate or narrow needle-like, or bimucronate at apex, margins are entire, and the colour ranges from bright green to reddish, based on light intensity and nutrient availability in the habitat. Seeds are oval, spherical or. Flowers are bracteate and bracteolate, with calyx lobes 3-5, small, corolla lobes 3-5 or absent in some, and nectar scales are seen in most species. Stamens 3-5, inserted at the base of the calyx tube. Ovary ellipsoid, style short, stigma capitate, capsule globose, seeds ovoid, brownish, 10-15 per fruit. The seed micromorphological features of *Rotala* have not been fully explored so far; hence, the present study mainly focused on the seed coat characterization of selected species of *Rotala* from India to fill this gap.

METHODOLOGY

COLLECTION OF SAMPLES

Rotala species were collected from various locations in Kerala for seed morphological studies from June 2024 to January 2026 (Table 1). The plant taxa selected for the present study include *R. cheruchakkiensis*, *R. densiflora*, *R. indica*, *R. kasaragodensis*, *R. macrandra*, *R. mabarica*, *R. mulanwuzhensis*, *R. meenkulumensis*, *R. mexicana* and *R. occultiflora*. Identification of selected taxa was done by observing their morphological characteristics with the help of available floras and literature and it was also compared with authenticated herbarium specimens deposited in the herbarium of University of Calicut (CALI), the voucher specimens were also deposited in the herbarium of St. Joseph's College (Autonomous), Devagiri, Calicut (DEV) for future reference. Fresh plant specimens were preserved in formalin and FAA for further morphological characterization.

The plant species investigated in this study were collected from their natural habitats within the study region, based on the availability of material. To obtain seeds, mature seeds of *Rotala* plants were removed from fruits, collected during field visits, in the fruiting season. Seed debris were removed by treatment with distilled water, followed by air drying. A detailed list of the plant species studied, along with their collection sites and voucher numbers, is provided in Table 1. In total, 10 species of *Rotala* were investigated. From each species, ten seeds were analyzed and evaluated using the most employed morphological descriptors (Table 2).

LIGHT MICROSCOPIC ANALYSIS OF SEEDS

Mature seeds of plants were repeatedly washed with ethanol to dissolve waxes, debris and also to sterilize surface. External colour, shape, size (length and width), and hilum were observed by using a stereo microscope (Olympus SZX2ALLT) equipped with a Magnus Magma DC 10 camera, placed in Plant Anatomy Lab Department of Botany, Govt. College, Kasaragod, Vidyanagar, Kerala, India. Seed shape was identified by Seed Identification Manual (Alexander C. Martin & William D. Barkley, 1961).

SCANNING ELECTRON MICROSCOPIC ANALYSIS OF SEEDS

Seed samples were prepared for the SEM study following the protocol of Usma et al, (2019). using SEM (Model JEOL/FE, VERSION 4, JSM IT 800) ornamentation of seed has been observed. Collected seeds were separated from plant immediately on stubs by using tape. These samples were then sputter-coated with gold—palladium and examined by SEM. The examined specimens were identified and snapped by Polaroid P/N 665 film, The diagnostic features of seed coat such as seed shape and surface, testa features (Cie, epidermal cells and cell wall pattern) were described according to the terminologies of (Barthlott, 1981). In all cases, seeds of 10 samples of each species were analyzed,

ANALYTICAL TECHNIQUES FOR FESEM AND EDAX ANALYSIS

Field Emission Scanning Electron Microscopy (FESEM) is employed to explore the micro structural characteristics of *Rotala* species in conjunction with Energy Dispersive X-ray Analysis (EDAX) to delineate the elemental composition of its seeds. The imaging provided by FESEM facilitates high-resolution of seed coat morphology; whereas the EDAX analysis permits the identification of critical minerals. Through this comprehensive methodology, we aspire to elucidate the structural and elemental features that are fundamental to the plant's ecological resilience and pharmacological capabilities, thereby offering significant contributions to the fields of conservation biology,

CHEMICALS AND SAMPLE PREPARATION

In this investigation, a diverse array of chemical agents was employed to prepare seed samples of

Rotala species for Field Emission Scanning Electron Microscopy (FESEM) and Energy

Dispersive X-ray Spectroscopy (EDAX) analyses, thereby enhancing the clarity and resolution of specific structural and elemental features,

SPUTTER-COATING TECHNIQUE

To facilitate the preparation of samples for FESEM analysis, a sputter-coating technique was implemented, which necessitated the uniform deposition of a thin layer of gold or platinum onto the surfaces of the samples. This conductive coating effectively mitigated charging artifacts and enhanced image quality under high vacuum conditions, thereby enabling precise visualization of surface characteristics such as trichomes and cellular textures at the microscopic scale. Regarding EDAX profiling, no supplementary chemicals were directly introduced to the samples, as this technique is predicated on the detection of characteristic X-rays emitted from the elemental constituents of the samples when subjected to an electron beam bombardment. This analytical approach permitted a non-destructive evaluation of vital minerals including carbon (C), oxygen (O), calcium (Ca), magnesium (Mg), potassium and trace metals, which are pivotal for elucidating the elemental composition of seed coats of *Rotala* species. The integration of these chemical preparations and analytical methodologies facilitated a holistic understanding of the seed coat surface and elemental characteristics of the plant, bolstering insights into its prospective medicinal and ecological adaptations. In the current study, seed coat morphology and FESEM-EDAX of ten seeds of *Rotala* species was studied, *R. cheruchakkiensis*, *R. densiflora*, *R. indica*, *R. kasaragodensis*, *R. macrandra*, *R. malabarica*, *R. malampuzhensis*, *R. meenkulamensis*, *R. mexicana* and *R. occultiflora* (Table I). Images of studied *Rotala* species are shown in Fig. 1 & Fig. 2.

MORPHOMETRICS

Statistical research based on the seed morphological traits of the studied taxa was performed BY morphometric methods. Traits, including seed color, shape, sculpture, hilum shape, seed length, seed width, seed L/W ratio, trichomes were performed. Five measurements were made of each seed's width and length. The length-to-width ratio is calculated using the LAV formula, where L is the seed length, W, is its width (Table. 2).

Table 1 . Collecting data on the *Rotala* species selected for the seed coat micromorphology and FESEM-EDAX studies.

No.	Taxa	Habitat	Geographical coordinates	Details of plant specimens studied
	<i>R. cheruchakkiensis</i> Anto Pev., Devikrishna C.S., Jacob Abraham Pulickal. Vraghese C. D, and Ignatius Anton	wet lateritic soil	12 28 75 23 so ⁱ	Karinthalam, Kasaragod District. Kerala State, Suvarnika.V., 10409, DEV, 17/07/2024
2	<i>R. densiflora</i> (Roth ex Roem. &Schult,) Koehne	Dry paddy field	1 10 84'67" 76 06 31'	Kattikkulam, Wayanad District, Kerala State, Suvarnika, V.. 10406, DEV, 10/01/2024
3	<i>R. indica</i> (Willd.) Koehne	Wet paddy field	12022 35" 75 04 28 ¹	Thannott, Ravaneshwars Kasaragod District, Kerala State, Suvarnika.V., 10410, DEV, 13/1 1/2025
4	<i>R. kasaragodensis</i> K.S. Prasad & Raveendran	Seasonal lateritic pool	12 1425' 75 ⁰ 13'55" E	Velichamthodu, Cheer Districts Kerala State, Suvarnika.V., 10403, DEV, 13/09/2024
5	<i>R. macrandra</i> Koehne	Wet paddy field	12039'69"N 75°39'69" E	Vellamthatta, Ravaneshwar, Kasaragod District' Kerala State, Suvarnika.V., 10408, DEV, 06/01/2025
6	<i>R. malabarica</i> Pradeep, K.T. Joseph &Sivar.	Seasonal lateritic pool	12 28 75 23	Karinthalam, Kasaragod District, Kerala State, Suvarnika.V., 10404, DEV, 10/10/2024
7	<i>R. malampuzhensis</i> R.V, Nâir ex C.D.K. Cook	Seasonal lateritic pool	12026'49"N 75 ⁰ 18'37" E	Kayyur, Kasaragod District, Kerala State, Suvarnika.V., 10402, DEV, 25/09/2024
8	<i>R. meenktdamensis</i> KS. Prasad & k. Ravi	Seasonal lateritic pool	12 28 87 ¹ 75 ⁰ 2380" E	MeenkuIam, Pyyannur Kannur District, Kerala State, Suvarnika.V., 1041 1, DEV, 15/10/2025
9	<i>R. mexicana</i> Schltdl. & Charm	Wet lateritie soil	12 1425 75013'55iiE	Kerala State, Suvarnika.V., 10405, DEV, 24/09/2024
10	<i>R. occulliflova</i> Koehne	Seasonal lateritic soil	12 28 75023 so ⁱ	Karinthalam, Kasaragod District. Kerala State, Suvarnika.v., 10403, DEV, 13/09/2024

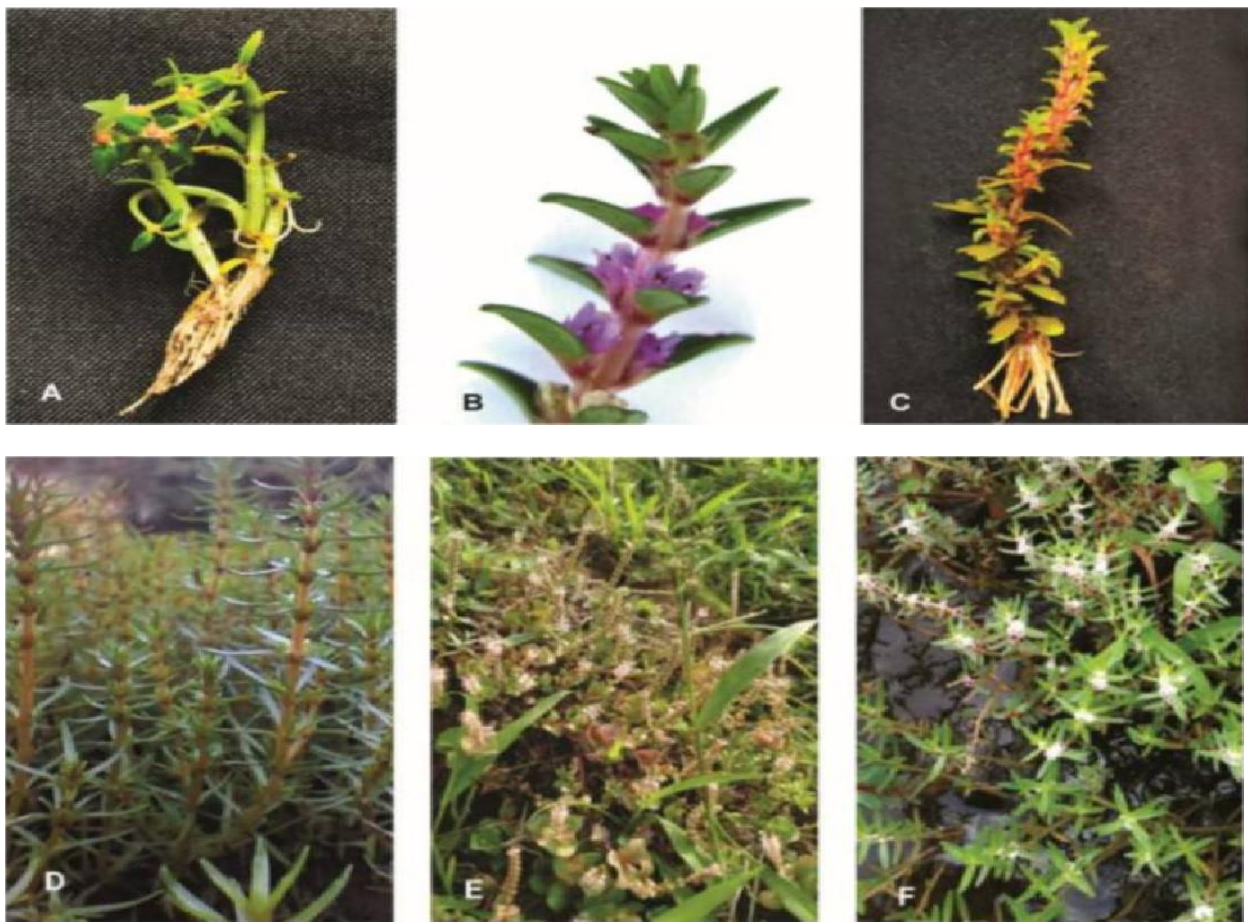


Fig. 1. Habit or selected *Rotala* spp. from the study area:

A: *R. cheruchakkiensis*; B: *R. densiflora*; C: *R. indica*; D: *R. kasaragodensis*; E: *R. macrantha*. F: *R. malabarica*



Fig. 2. Habit of selected *Rotala* spp. from the study area:
G: *R. malampuzhensis*; H: *R. malampuzhensis*; I: *R. malampuzhensis*; J: *R. malampuzhensis*

OCW<if10ru; J: R

RESULT

General seed coat characters of the genus *Rotala* are discussed below:

The dominant seed shape was ovate and obovate (fig. 3). Seeds of all the studied species were found with smooth surface and with terminal hilum. The seed of *R. occultiflora* was observed with a maximum length of 297.8 mm to width of 1 15.6 mm. Length to width ratio of seeds was also calculated (Table, 2), and it was found that the maximum L/W ratio was observed in *R. occultiflora* i.e., 2.576mm.

Table 2. Seed micro morphological characteristics of studied *Rotata* spp.

Species	Seed shape	Seed colour	Seed length	Seed width	Seed LAV ratio	Hilum	Trichome	Seed coat texture
cheruchakkiensis	Round elliptic	Golden brown	452.1 mm	334.8mm	1.350mm	Acute	Present	Reticulate
<i>R. densiflora</i>	Oval	Pale brown	440.5 mm	359.1 mm	1.226mm	Acute	Absent	Reticulate
<i>R. indica</i>	Elliptic	Pale brown	425.2 mm	335.4mm	1.267mm	Acute	Present	Reticulate Linear
<i>R. kasavagodensis</i>	Obovate	Pale brown	3.824 mm	3.022mm	1.265mm	Rounded	Absent	Wrinkled
<i>R. macrandva</i>	Obovate	Pale brown	374.0 mm	199.2mm	1.877mm	Acute	Absent	Reticulate
<i>R. malabarica</i>	Ovate	Dark brown	692.9 mm	600.2mm	1.154mm	Acute	Absent	Reticulate polygonal
<i>R. malampuzhensis</i>	Obovate	Dark brown	637.7 mm	518.4mm	1.230mm	Acute	Absent	Irregular polygonal
<i>R. meenku/amensis</i>	Ovate	Pale brown	670.3 mm	576.2mm	1.163mm	Acute	Absent	Irregular polygonal
<i>R. mexicana</i>	Elliptic	Pale brown	269.2 mm	170.4mm	1.579mm	Acute	Absent	Striate
<i>R. occultiflora</i>	Elliptic	Dark brown	297.5 mm	115.6mm	2.576mm	Acute	Absent	Striate

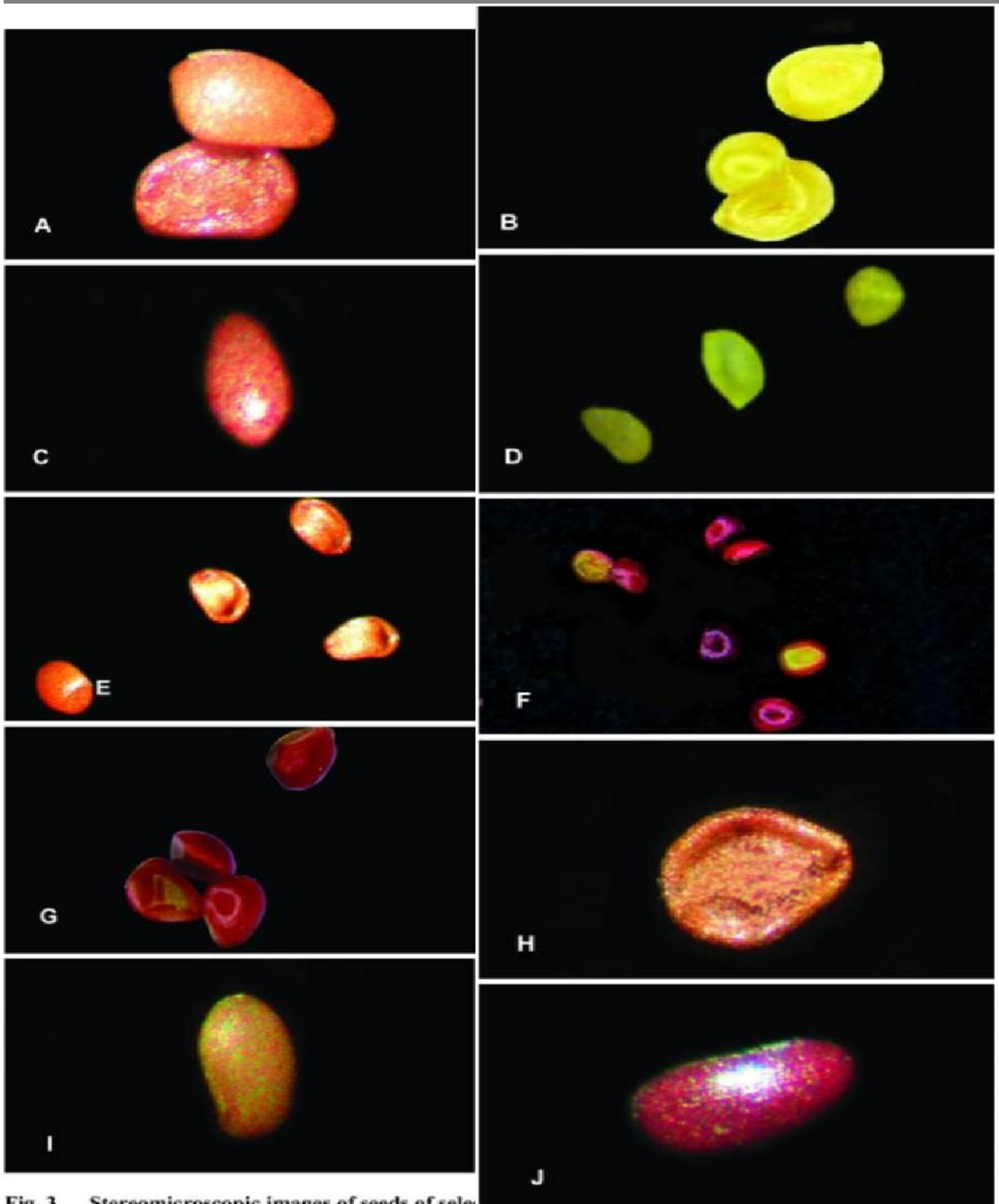


Fig. 3. Stereomicroscopic images of seeds of selected

Stereomicroscopic images of seeds Of selected *Rotala* spp.:

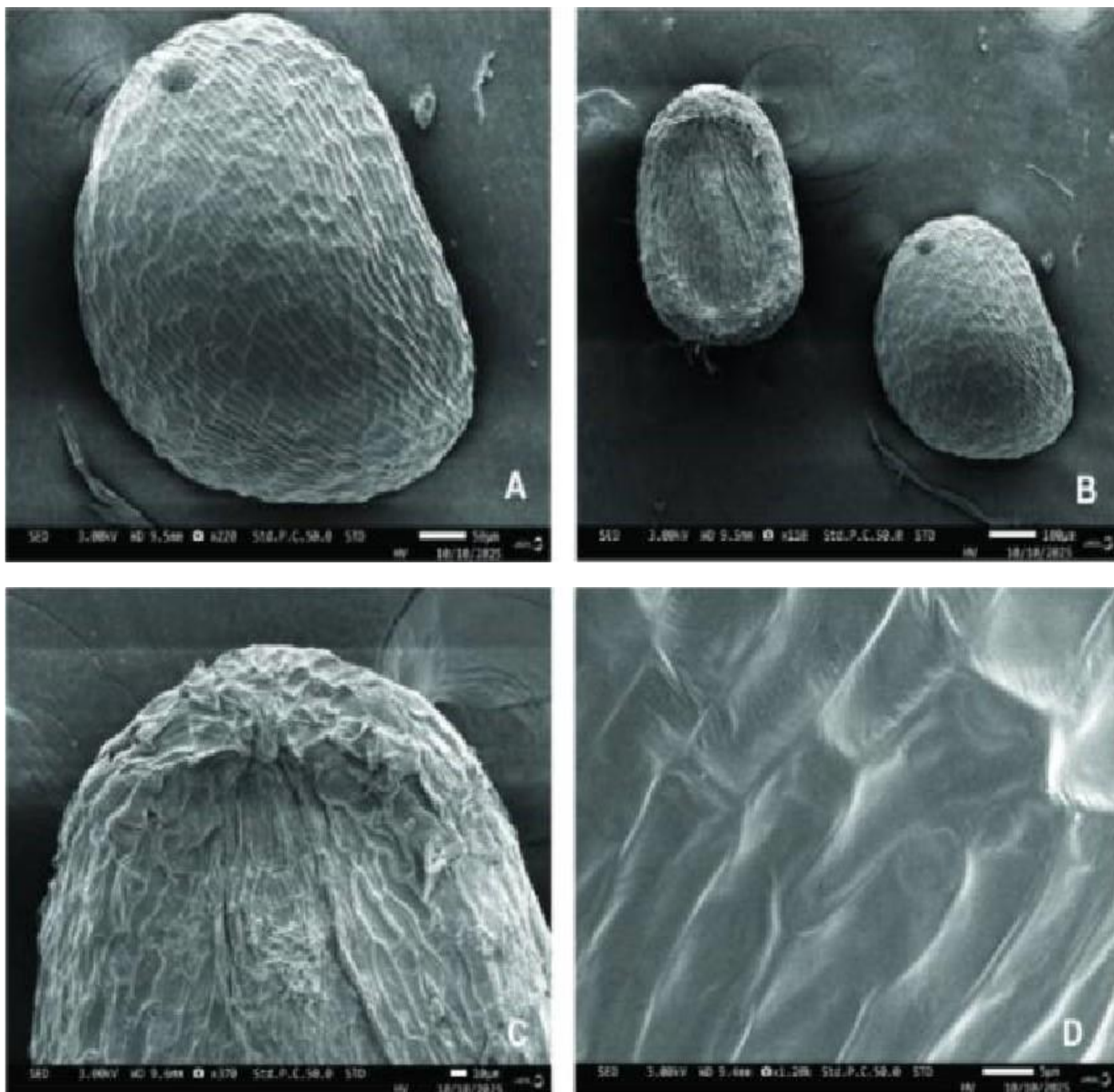
A: *R. cheruchakkiensis*; B: *R. densiflora*; C: *R. indica*; D: *R. kasaragodensis*; E: *R. macrandra*•,

F: *R. ma/abarica*; (i; *R. malampuzhenyis*; H; *R. meenktadamensis*; I: *R. mexicana*; J: *occultiflora*

I *R. cheruchakkiensis* Anto P. V. , Devikrishna C.S., Jacob Abraham Pulickal, Vraghesc C. D, and Ignatius Antony

R. cheruchakkiensis is the smallest species in the genus *Rotala*, with a height about 3m. Golden to brownish coloured seeds are seen. The number of seeds per fruit are 8-9. The seed shape was flattened oval and the texture was smooth. The compression was lateral, while the hilum was terminal. The length of the seed was 452.1 mm, whereas the width of the seeds was 334.8 mm. while length to width ratio of 1.350mm was recorded. Fig.(4) represent the FE-SEM results of *R. cheruchakkiensis*. that indicates smooth seed sculpturing with epiclimal hairs. Seed coat ornamentation is reticulate. Wings are absent on seed coat surface. Outer epidermal cell shape and anticlinal wall shape are irregular; anticlinal wall texture is folded. Anticlinal wall is thickened and its level is shallow. Periclinal wall is thickened, convex shaped and its texture is reticulate,

The morphological variation of the seeds seems to reflect the geographical distribution of taxa, but adaptation may have played an important evolutionary role. *R. cheruchakkiensis* considered as highly endemic, it emerges during first rainy seasons of the month June and completes its life cycle within 1-2 months.



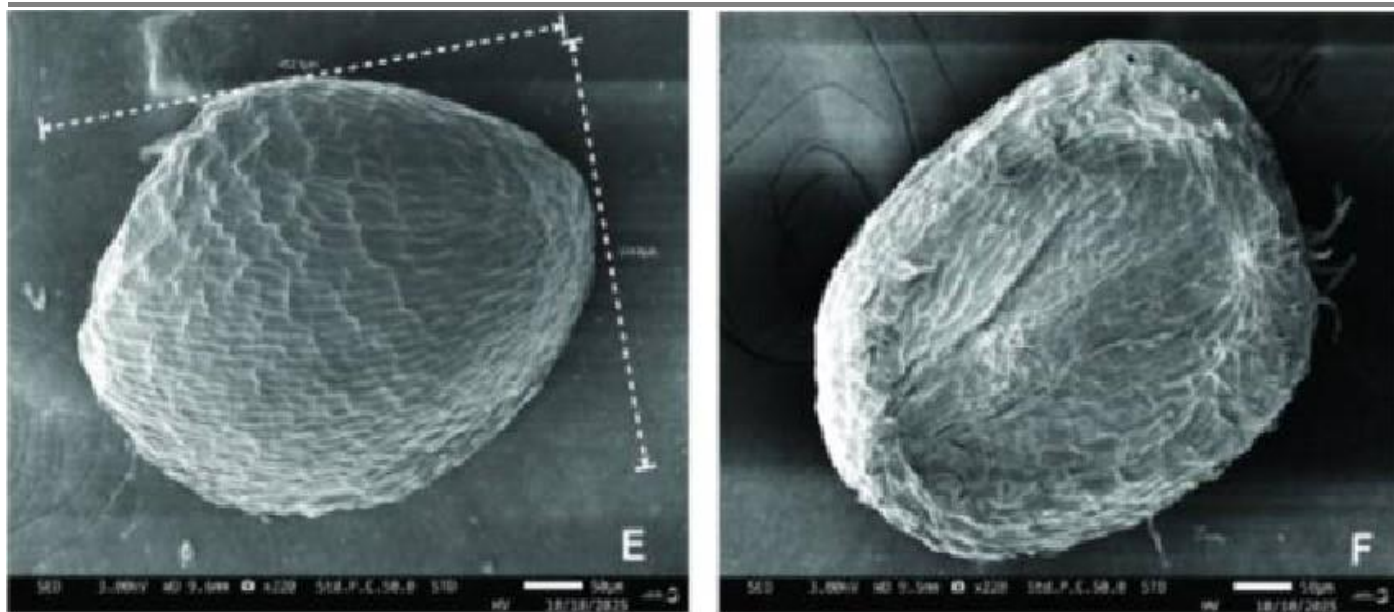


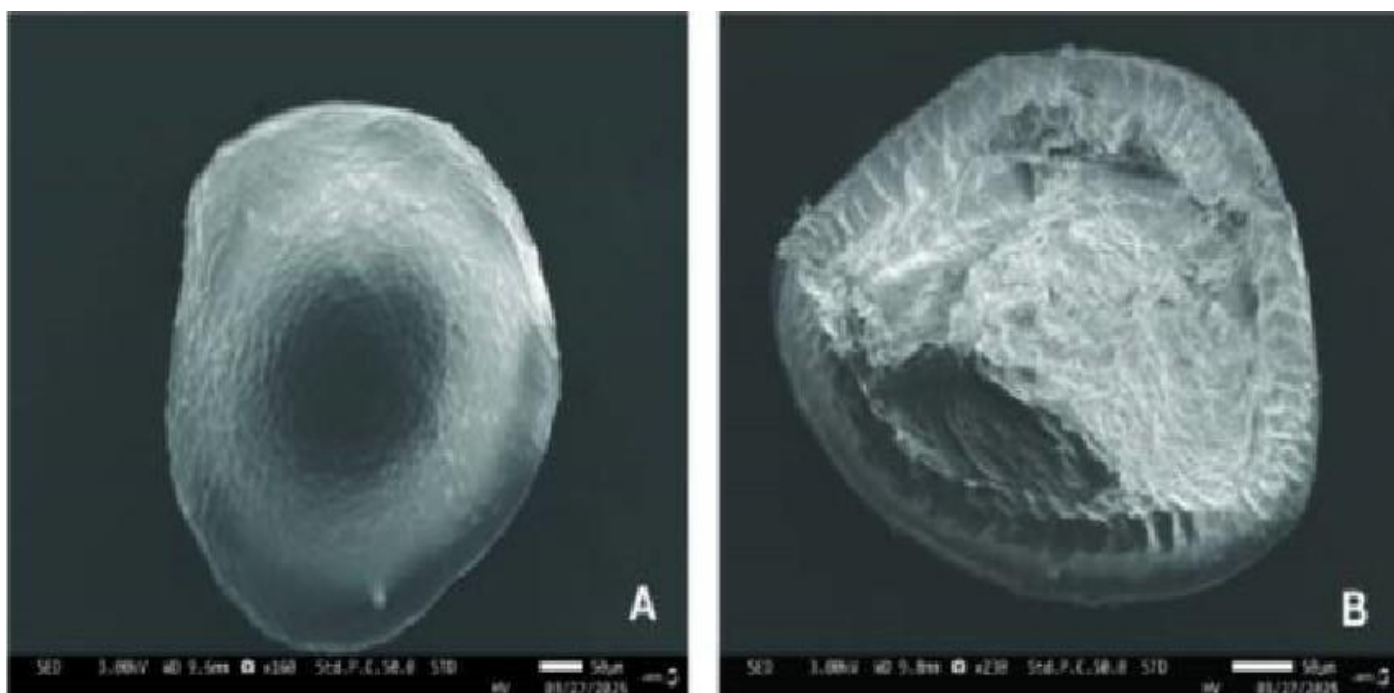
Fig. 4: SEM micrographs of seed coat surface (scale bar—10µm). A: face; B: raphe and hilum; C: seed coat surface; D: seed coat surface; E: seed coat surface; F: seed coat surface.

Length and width of seed

Length and width of seed

1.2. *R. densifloro* (Roth ex Roem. &Schult.) Koehne

R. densifloro is usually growing in paddy fields and marshy areas. Stem is highly branched and produces dense flowers towards the stem apex. The number of seeds per fruit are 10-12, which are pale brown in colour. The seed shape was triangular ovoid and the texture was smooth. The hilum was terminal. The length of the seed was 440.5 mm, whereas the width of the seeds was 359.1 mm, while length to width ratio was 1.226mm. Fig.(S) represent the FE-SEM images of *Rx densiflora*, seed sculpturing or seed coat ornamentation was reticulate and without epidermal hairs. Wings are absent on seed coat surface. Outer epidermal cell shape and anticlinal wall shape are irregular; anticlinal wall texture is folded. Anticlinal wall is thickened and its level is shallow. Periclinal wall is thickened, convex shaped and its texture is reticulate.



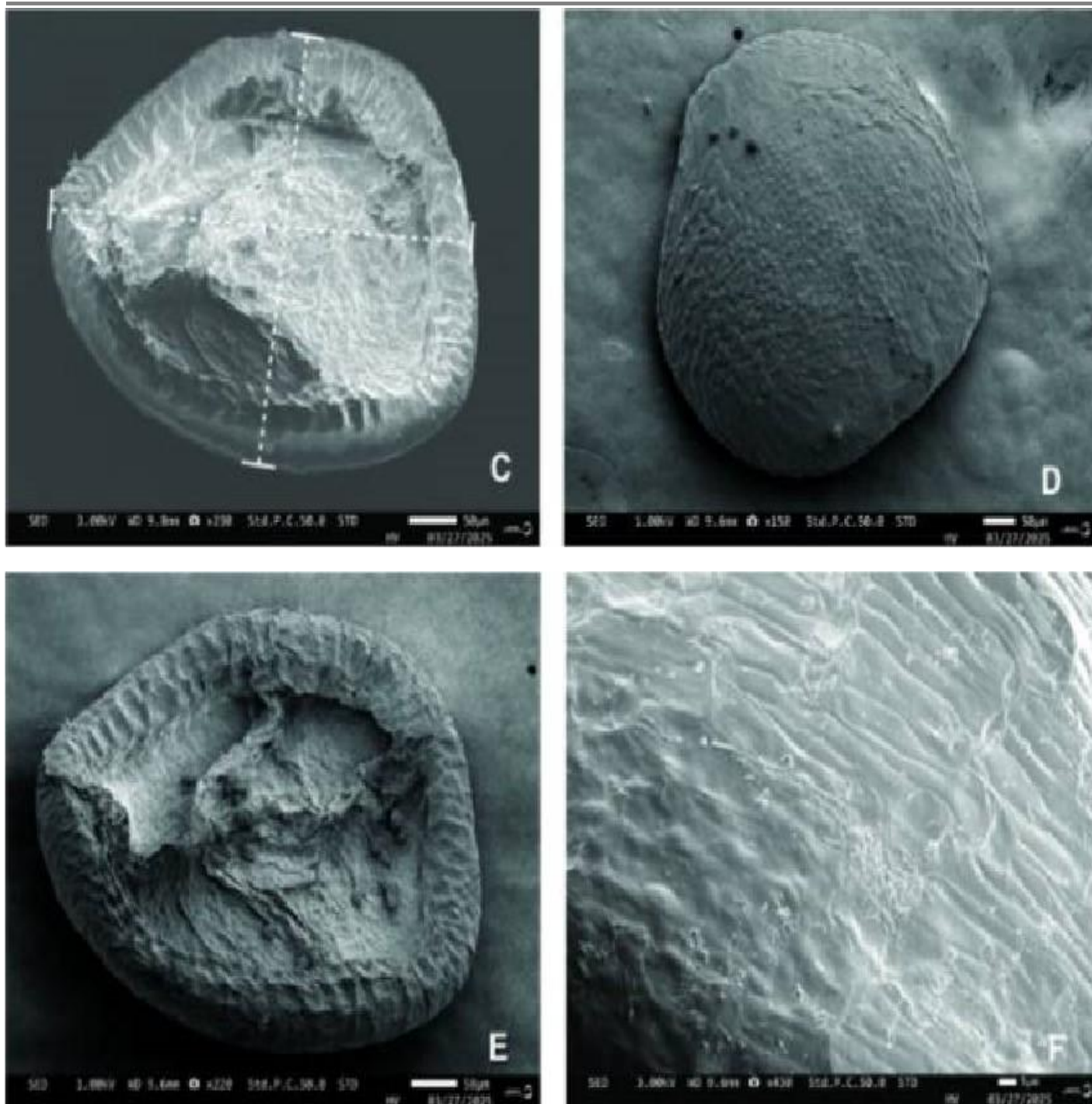


Fig. 5. SEM micrographs of seeds of *R. densiflora* (scale bar— 5µm & 50µm). A&D: antiraphal face; B&E: raphal face; C: length and width" F: seed coat surface.

1.3. *R. indica* (Willd.) Koehne

R. indica in habits paddy field and temporary lateritic pools, The plants may have white to reddish colour according to the habitat. Flowers are light rose in colour. The number of seeds per fruit are 10- 12. The seed shape was oval; seed coat ornamentation reticulate. The compression was lateral, while the hilum was terminal. The length of the seed was 425.2 mm, whereas the width of the seeds was 335.4 mm, while length to width ratio of 1267mm was recorded. Fig.(6) represent the FE-SEM results of *R. cheruchakkiensis*, that indicates smooth seed sculpturing with epidermal hairs. Seed coat ornamentation is reticulate. Wings are absent on seed coat surface. Outer epidermal cell shape was polygonal; anticlinal wall texture was reticulate. Anticlinal wall was thickened and shallow. Periclinal wall was thickened with reticulate texture; convex level. Trichomes present on seed coat.

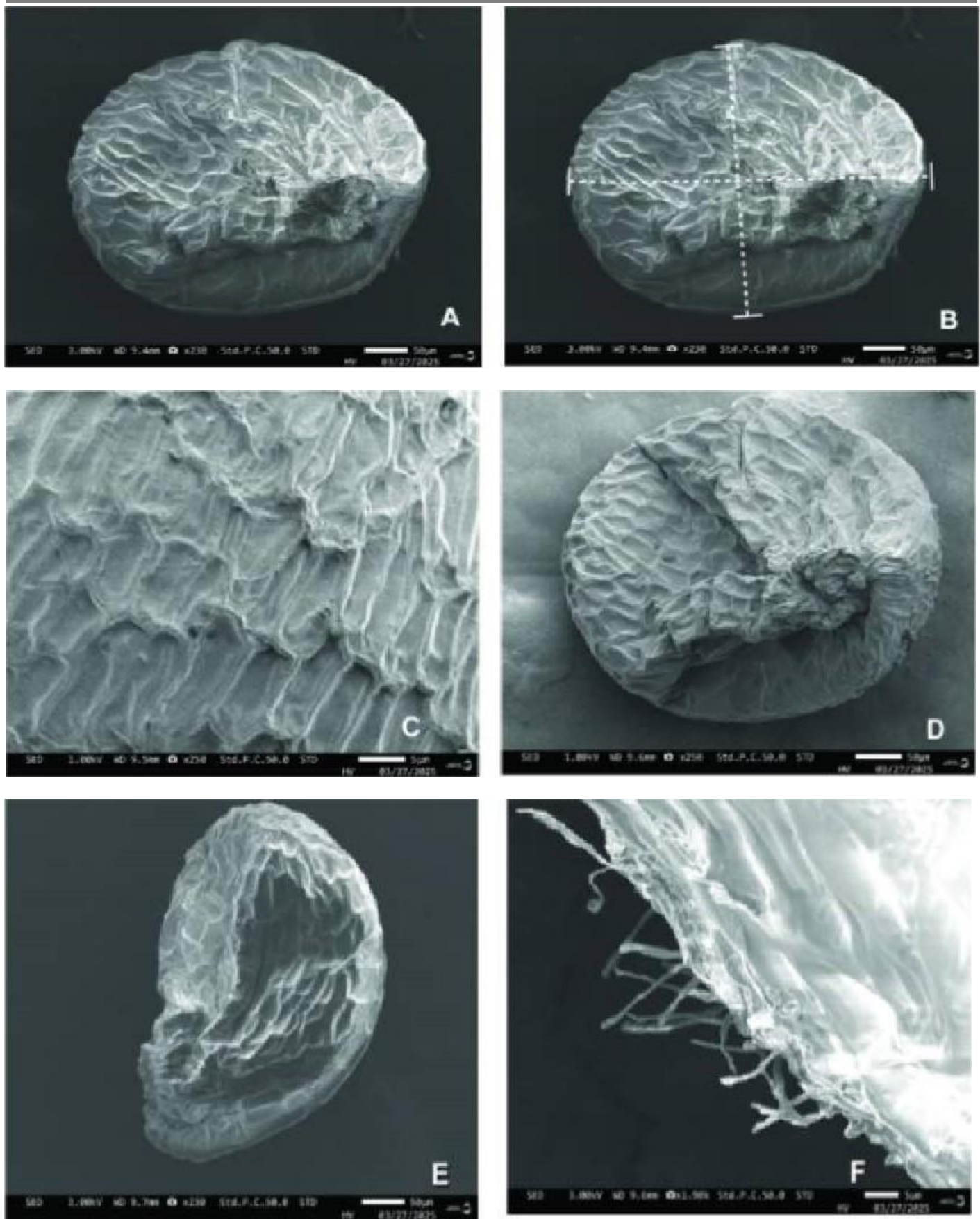


Fig. 6. SEM micrographs of seeds Of *R. indica* (scale bar= Sym & SOprn).

A&D: antiraphal face; B: length and width; C: seed coat surface; F: hilum

F; seed coat surface with evaginated trichomes,

1.4. *R. kasaragodensis* K.S. Prasad & Raveendran

R. kasaragodensis is usually found in the emergent marshy areas of temporary lateritic pools and paddy fields. Stem was erect and leaves arranged in cluster' Companulate, pink coloured flowers are produced singly from nodal regions, Leaves are narrow and needle like. Capsule was oblong with 10 ten seeds, which arc pale brown In colour. The seed shape was obovate and the texture was wrinkled. The hilum was terminal and rounded. The length of the seed was 3.824 mm, whereas the width of the seeds was 3.022 mm, while length to width ratio of 1.265mm was recorded (Fig.(4)). Epidermal hairs usually absent. Seed coat ornamentation was wrinkled, Wings are absent on seed coat surface. Outer epidermal cell shape and anticlinal wall shape are irregular; anticlinal wall texture is folded, Anticlinal and periclinal wall are thickened.

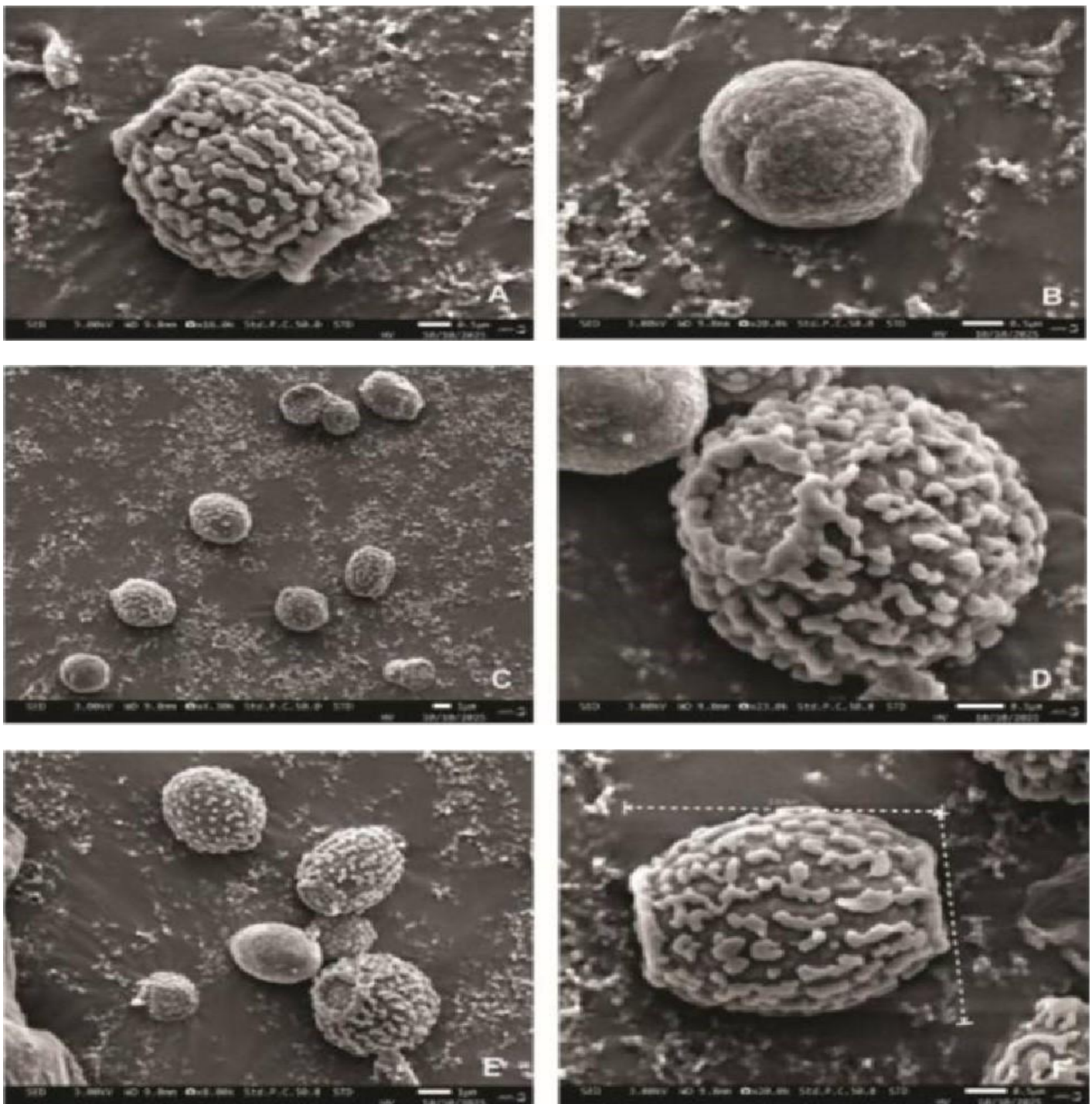
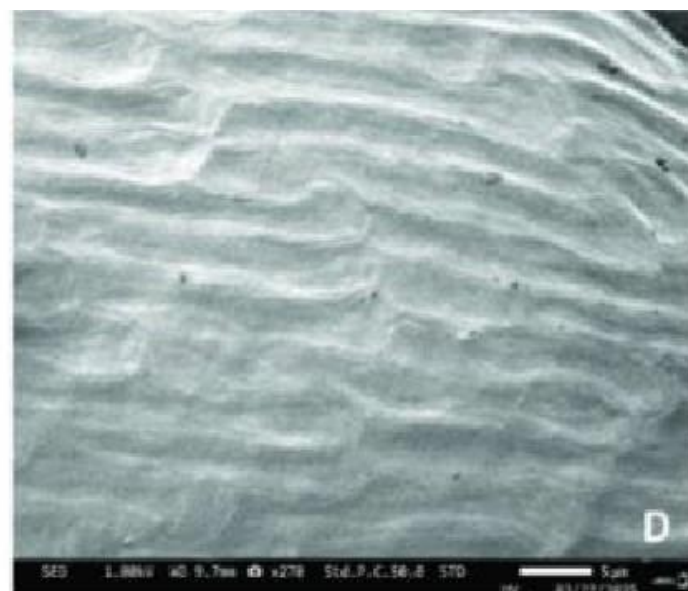
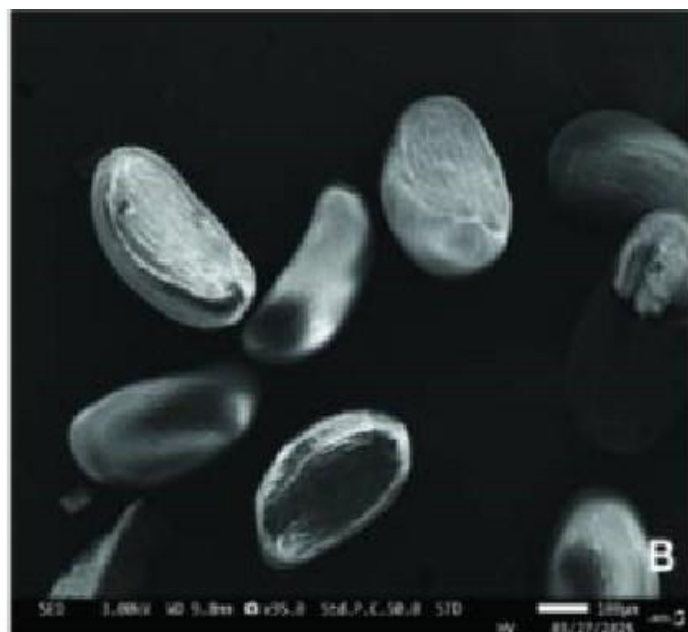
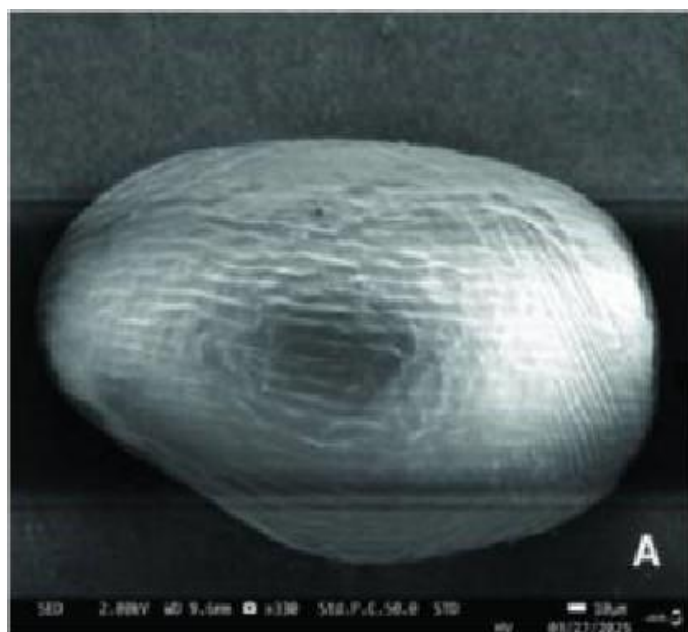


Fig. 7. SEM micrographs of seeds of *R. kasaragodensis* (scale bar— 50µm). A_E: raphal and antiraphal face; F: length and width.

1.5. *R. macrandra* Koehne

R. macvandra is growing in lateritic pools and marshy areas, Dimorphic leave are seen in this species; stem is white to reddish in colour. Flowers are pink and anthers exceeds the corolla tube. Golden to brownish coloured seeds are seen. The number of seeds per fruit are 8-9. The seed shape was obovate and seed colour was pale brown. Hilum was terminal. The length of the seed was 374.0 mm, whereas the width of the seeds was 199.2mm, while length to width ratio of 1.877mm was recorded. Fig. 4. represents the FE-SEM results of *R. macrandra*. Seed coat surface shows reticulate ornamentation. Epidermal hairs or trichomes are absent. Seed coat ornamentation is reticulate. Wings are absent on seed coat surface. Outer epidermal cell shape and anticlinal wall shape are irregular; anticlinal wall texture is smooth, Anticlinal wall is thickened and its level is shallow. Periclinal wall is thickened; convex shaped.



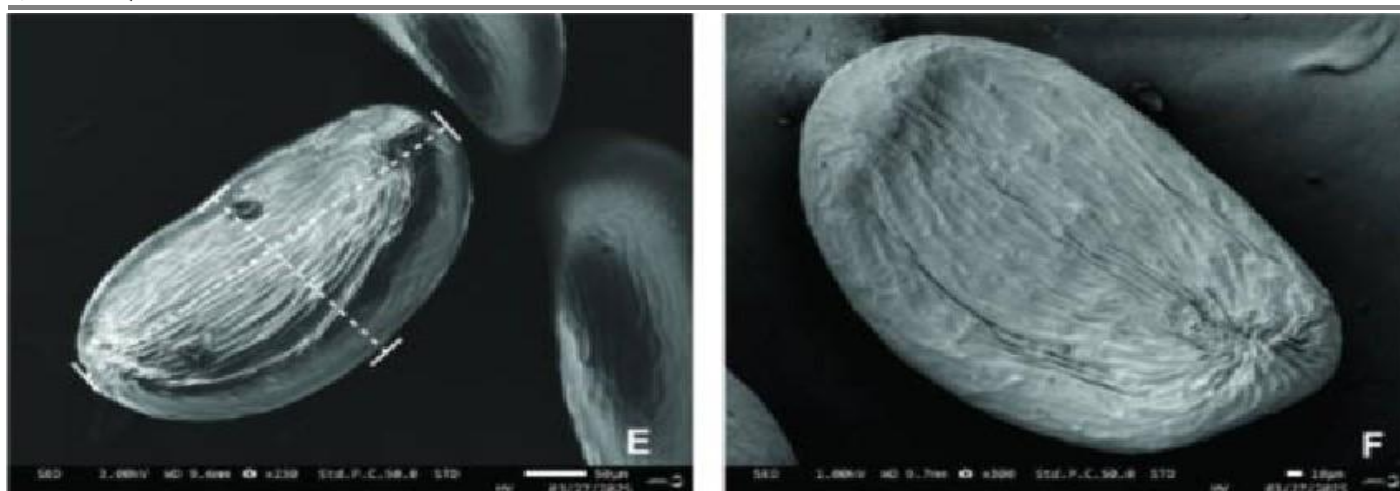
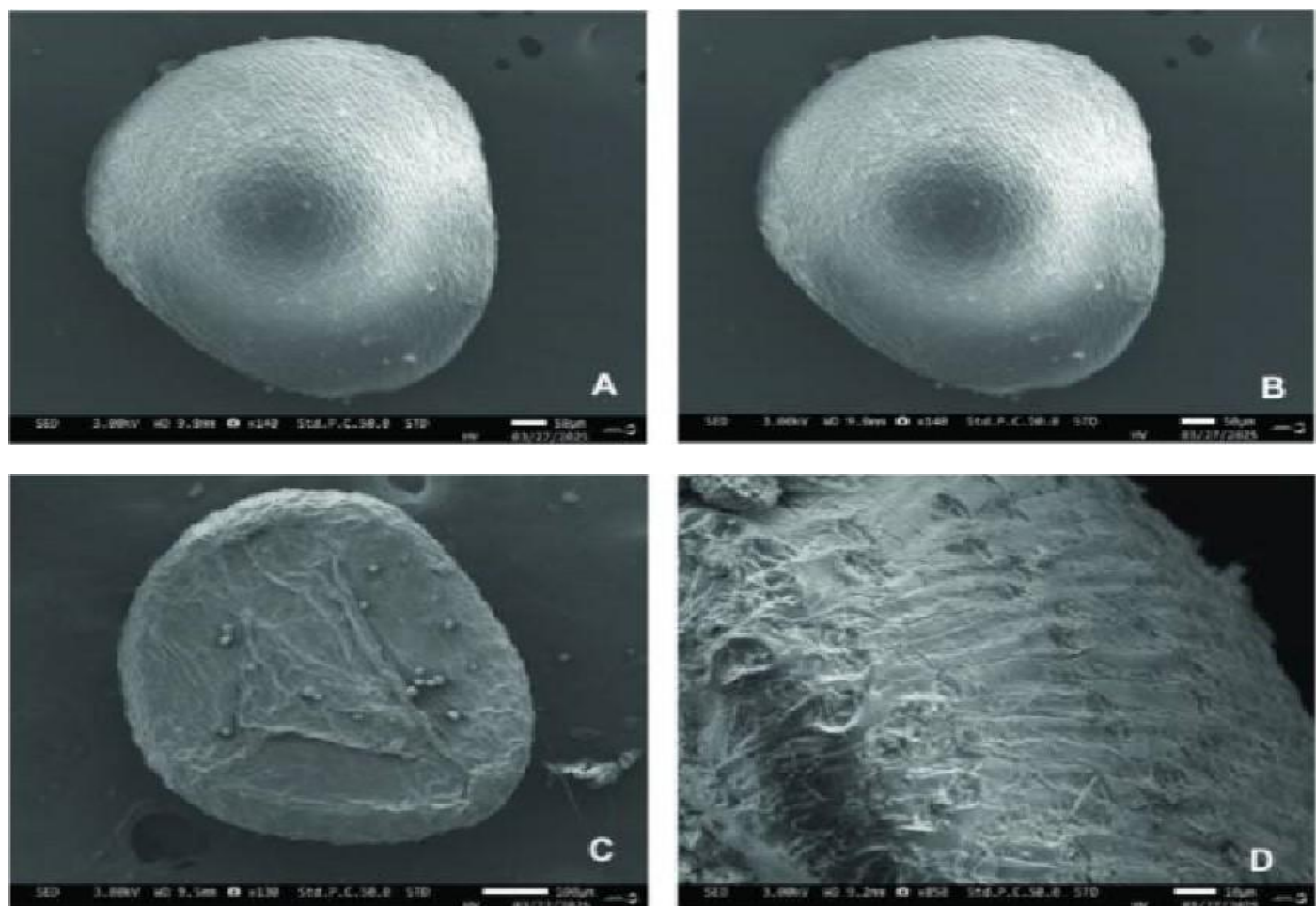


Fig. 8. SEM micrographs of seeds of *R. ntarandru* (scale bar— 5prn, IOpm, 50prn & IOOpm). A: antiraphal face; B: raphal and anti raphal face; C&F antiraphal face; D: seed coat surface; E: length and width

R, ma/abarica Pradeep, K. T, Joseph & Sivar.

R. malabarica is a herbaceous plant growing in seasonal lateritic pools. Its stem is quadrangular, leaves bimucronate. flowers are pentamerous. Seeds are dark brown in colour. The number of seeds per fruit are 10-12. The seed shape was oval and the texture was smooth. The compression was lateral, while the hilum was terminal. The length of the seed was 692.9 mm, whereas the width of the seeds was 600.2 mm, while length to width ratio of 1.154mm was recorded. Fig. 9&10 represents the FE-SEM results of *R. malabarica*. Seed coat surface is irregular polygonal. Wings are absent on seed coat surface. Outer epidermal cell shape and anticlinal wall shape are irregular; anticlinal wall texture is smooth. Anticlinal wall is thickened and its level is shallow. Periclinal wall is thickened, convex shaped and its texture is reticulate.



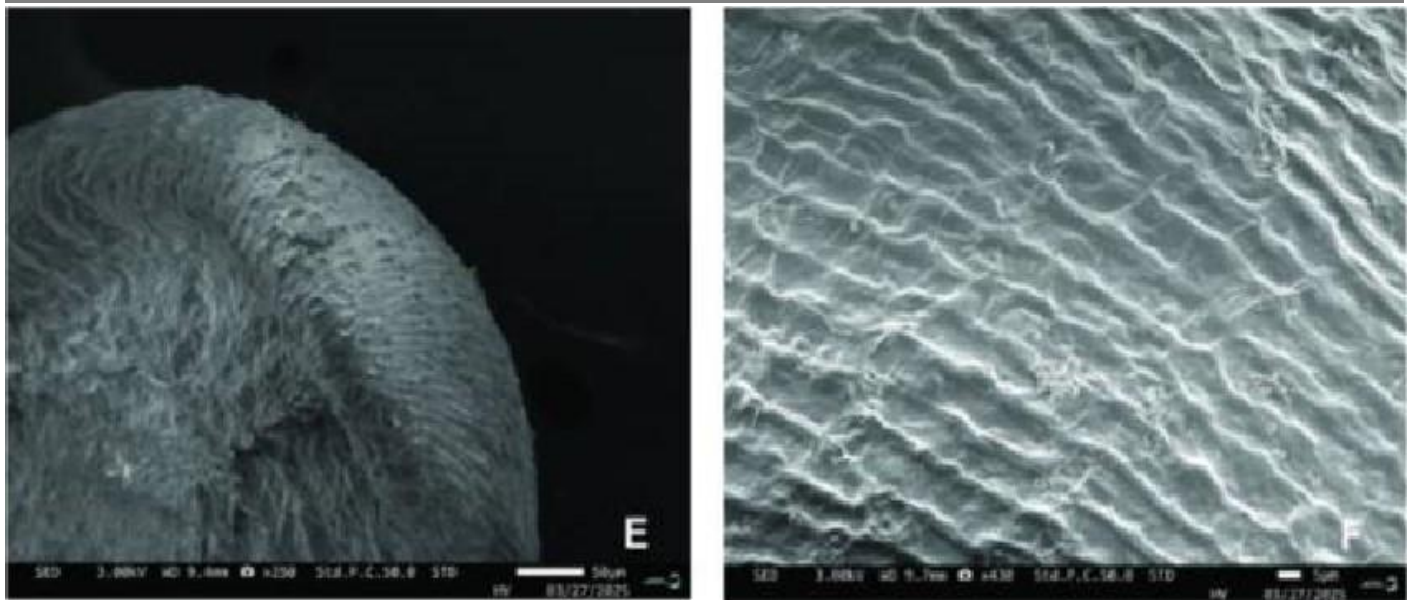


Fig. 9. SEM micrographs of seeds of *R. malabarica* (scale bar— 5pm, IOpm, 50pm & 10011m). A.&B: antiraphal face; C: raphal face; D.E&F: seed coat surface.

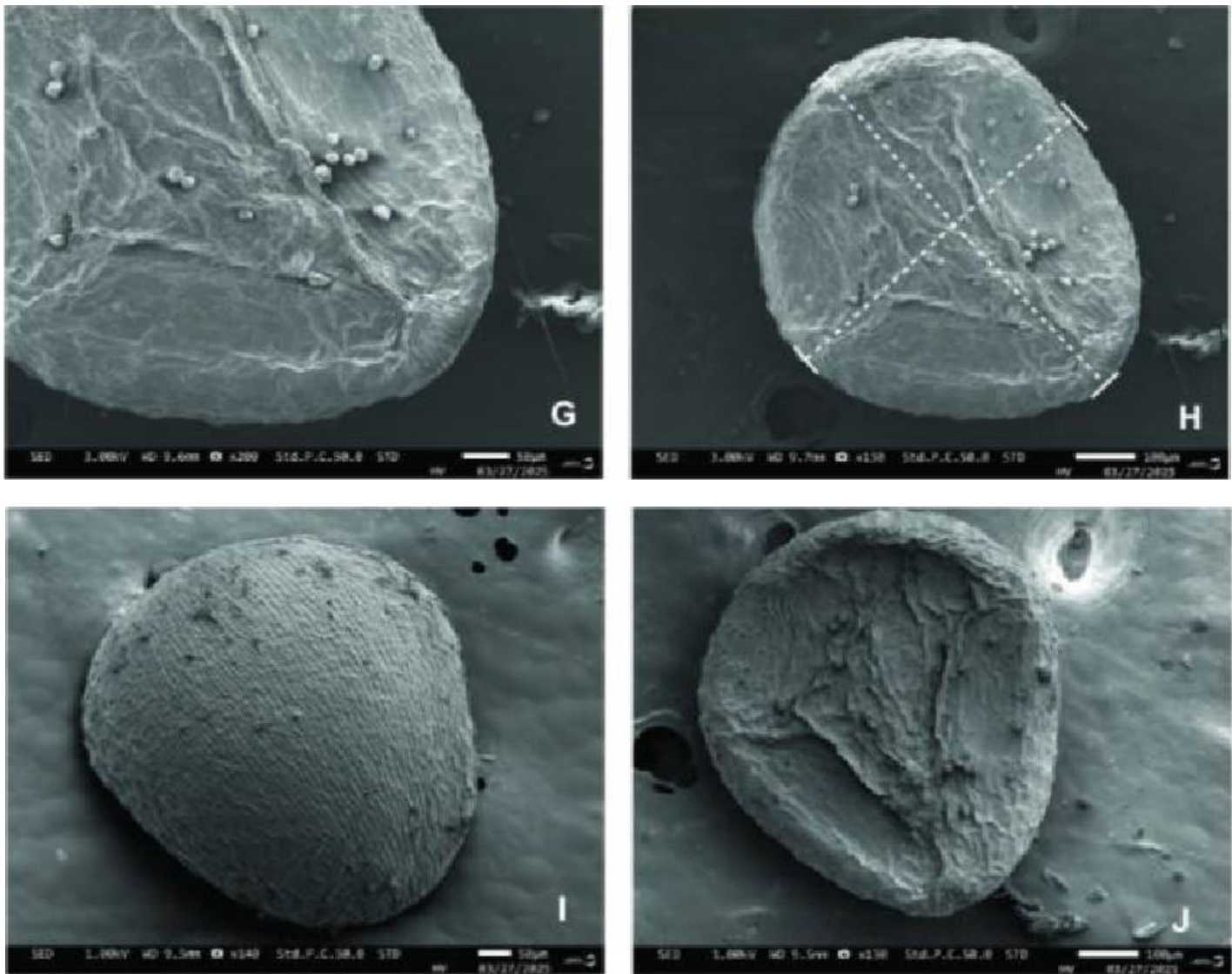


Fig. 10. SEM micrographs of seeds of *R. mulabarica* (scale bar= 50pm & IOOpm). G: hilum; H: length and width; I: antiraphal face; J: raphal face, malampuzhensis R-v. Nair ex C.D.K. Cook

R. malampuzhensis is growing in seasonal lateritie pools. Its stem is cylindrical, leaves lanceolate, flowers are tetramerous. Seeds are dark brown in colour- The number of seeds per fluit are 8-10. The seed shape was obovate and the texture was smooth. The hilum was terminal and acute. The length of the seed was 637.7mm, whereas the width of the seeds was 518.4 mm, while length to width ratio of 1.230mm was recorded. Fig. II represents the FE-SEM results of *R. malampuzhensis*. Seed coat surface is irregular polygonal. Wings are absent on seed coat surface. Outer epidermal cell shape is irregular polygonal and anticlinal wall is thin -'texture is smooth. Periclinal wall is thick, convex and its texture is reticulate. Trichomes are absent on seed coat surface.

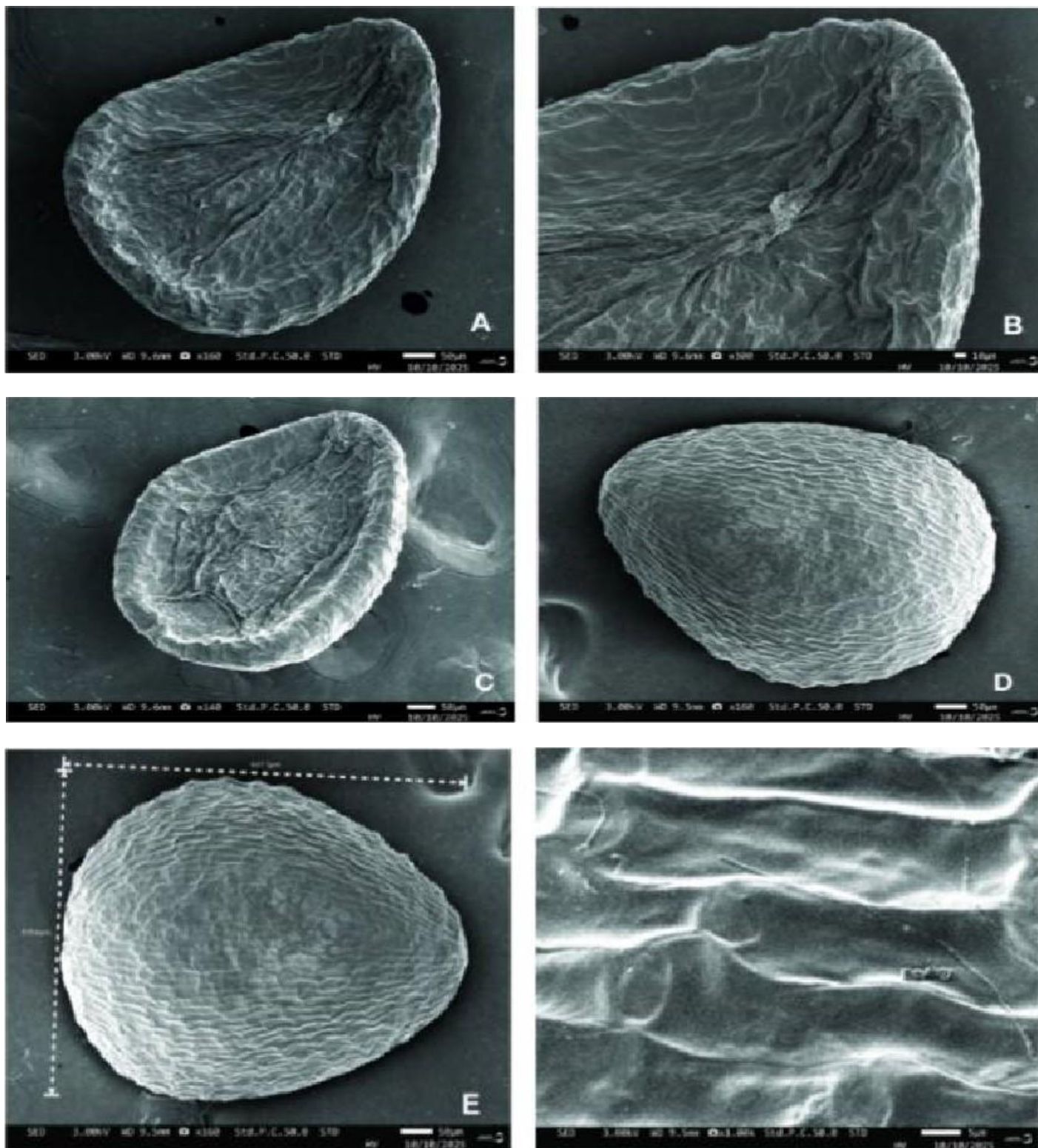
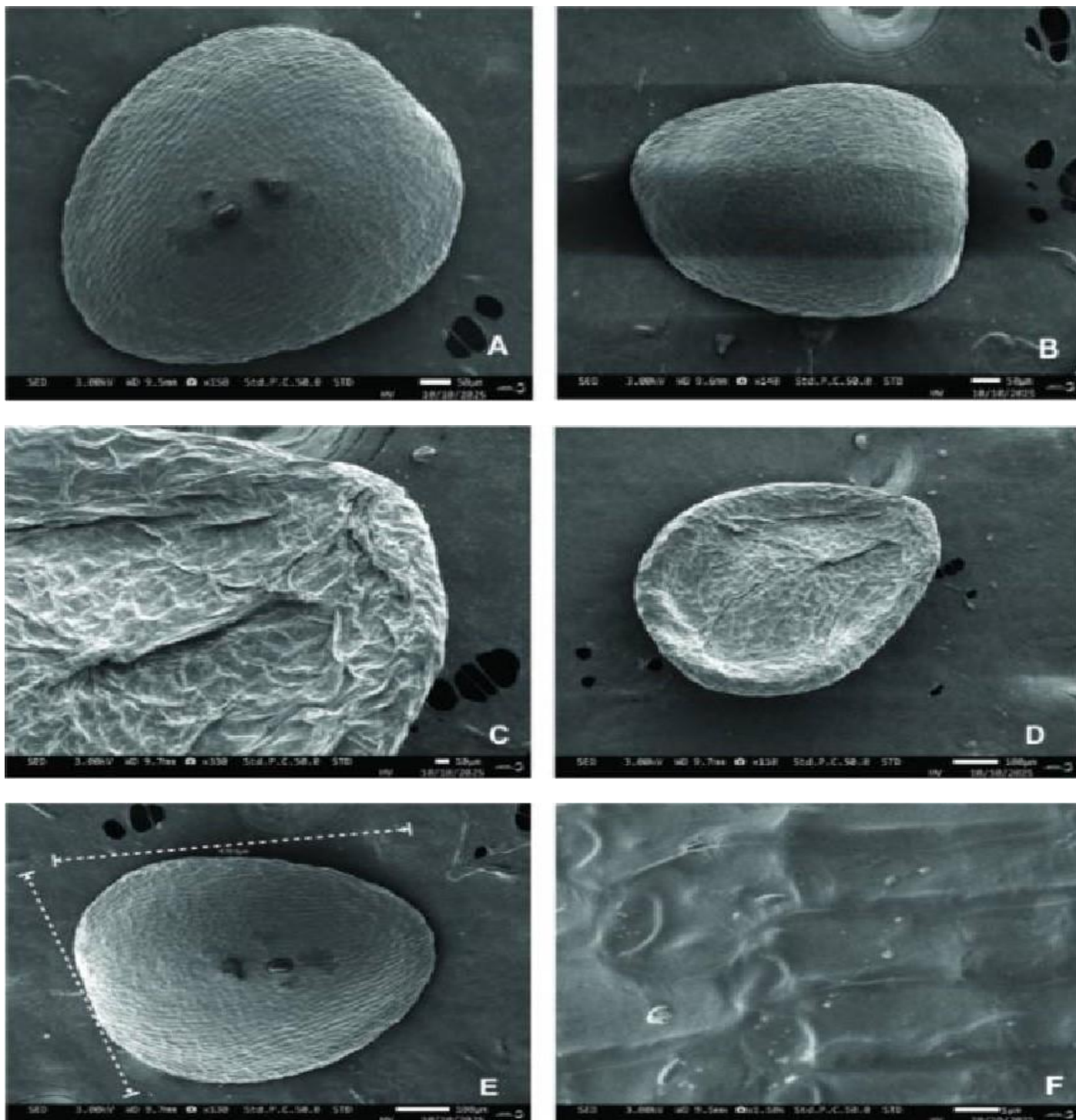


Fig. II. SEM micrographs of seedsof *R. malampazhensis* (scale 10 pm & 50pm). A&C: antiraphal face. B: hilum; D: E: length and width F: seed coat surface meenu/amensis K.S. Prasad & K. Ravi

R. meenkladamensis is a highly endemic species growing in seasonal lateritic pools with high humus content. Its stem is quadrangular and flattened at the base; flowers are pentamerous. Seeds are pale brown in colour. The number of seeds per fruit are 10-12. The seed shape was oval and the texture was smooth. The hilum was terminal. The length of the seed was 670.3mm, whereas the width of the seeds was 576.2 mm. while length to width ratio of 1.163mm was recorded. Fig. 12 represents the FE-SEM results of *R. meenkulamensis*. Seed coat surface is irregular, Wings and trichomes are absent on seed coat surface. Outer epidermal cell shape and anticlinal wall shape are irregular; anticlinal wall texture is smooth. Anticlinal wall is thin and its level is shallow. Periclinal wall is thickened, convex shaped and its texture is reticulate.



SEM micrographs of seeds of *R. meenkulamensis* A&B: antiraphal face; C: hilum; D: E: length and width F: seed coat surface mexicana Schtdl. & Cham

R. mexicana is a herbaceous plant growing in wet lateritie soils. Its stem is cylindrical, leaves leaneur, narrow and opposite in phyllotaxy. Flowers are Pink coloured. Seeds are elliptic and pale brown in colour.

The number of seeds per fruit are 10-12. The seed shape was oval and the texture was smooth. The compression was lateral, while the hilum was terminal. The length of the seed was 269.2mm, whereas the width of the seeds was 170.4 mm, while length to width ratio of 1.579mm was recorded. Fig. 13 represents the FE-SEM results of *R. mexicana*. Hilum is acuteseed coat surface is striate. Wings and trichomes are absent on seed coat surface. Outer epidermal cell shape and anticlinal wall shape are irregular; anticlinal wall texture is smooth. Anticlinal wall is thin and its level is shallow Periclinal wall is thickened; convex shaped

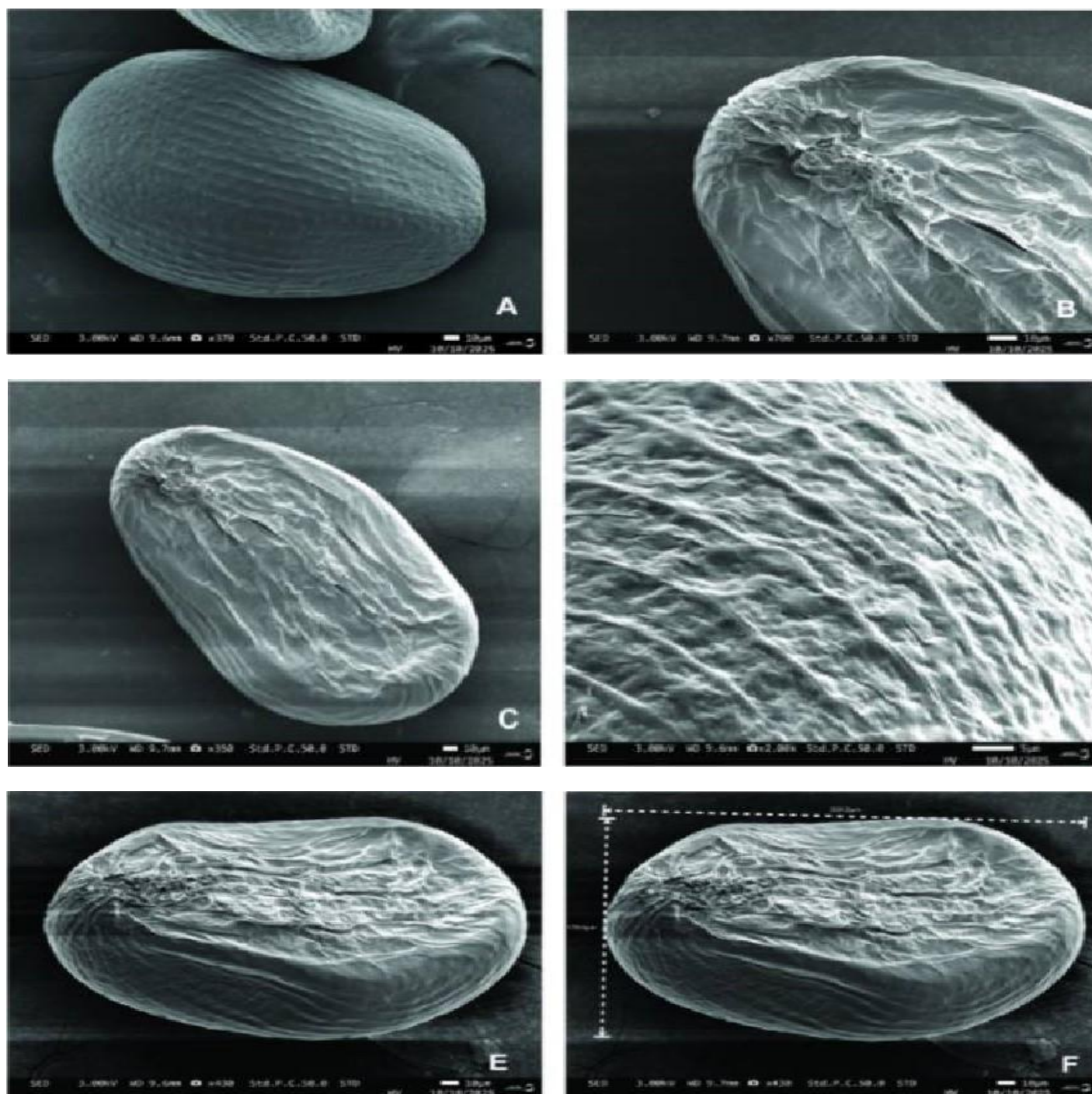


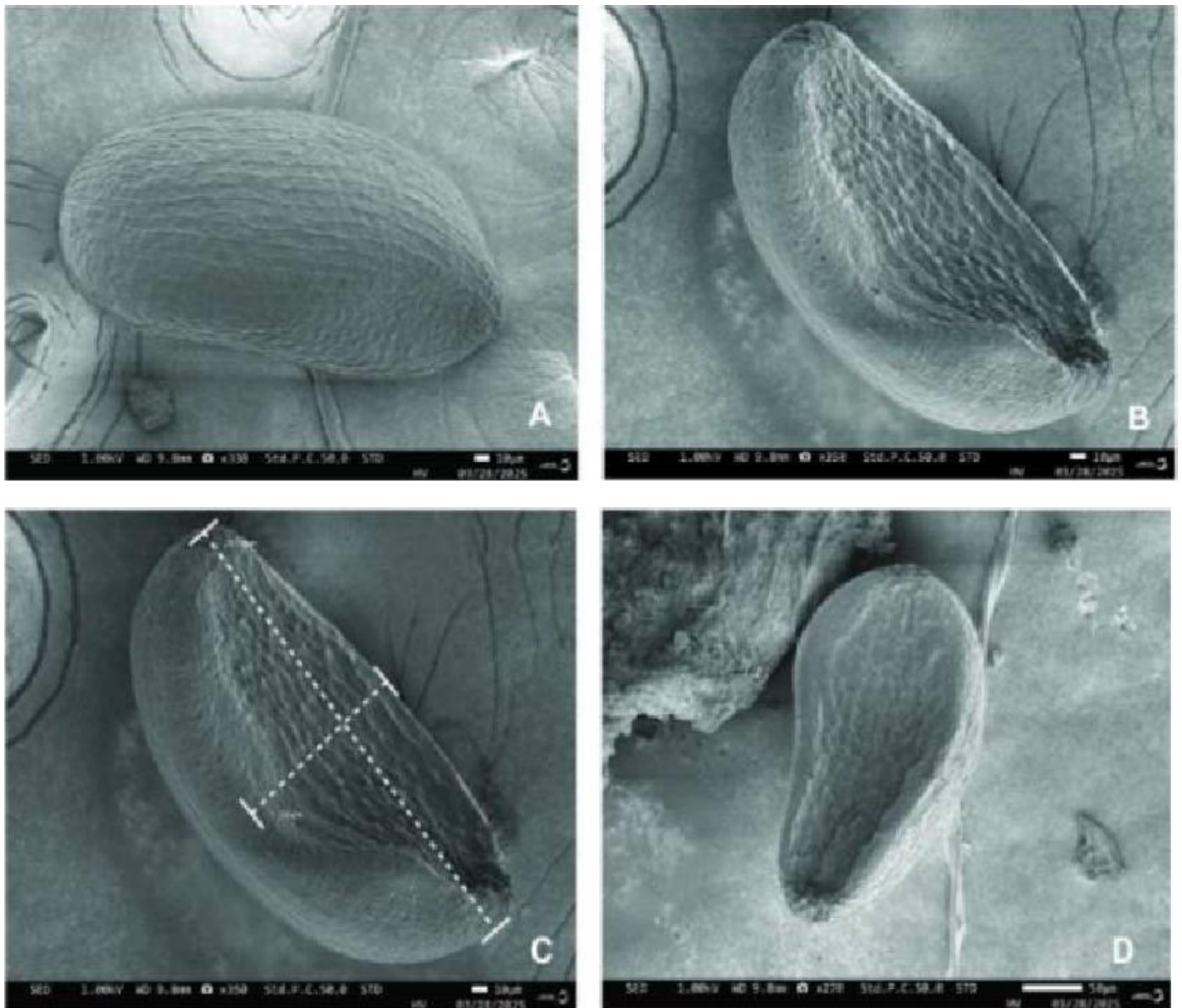
Fig. SEM micrographs Of seeds Of *R. mexicana* (scale bar= 5µm & 10µm).

A: antiraphal face; B: hilum; C&E: D: seed coat surface r: length and width

1 10.R. occultiflora Koehne

This plant is growing in lateritie seasonal pools. Stem is white to reddish according to the habitat.

Leaves are narrow and linear. Flowers are whitish, companulate with 4 lobes. Fruit is a capsule, 8-10 seeds per fruits seed shape is elliptical to pear shaped and dark brown in colour- The seed shape was oval and the texture was smooth. The compression was lateral, while the hilum was terminal. The length of the seed was 297.8mm, whereas the width of the seeds was 115.6 mm, while length to width ratio of 2,576mm was recorded, It shows the largest seed length to width ratio, among the studied species of Rota/a (Fig. 14). Hilum is acute,se.ed coat surface is striate. Wings and trichomes are absent on seed coat surface. Outer epidermal cell shape and anticlinal wall shape are irregular; anticlinal wall texture is smooth. Anticlinal wall is thin and its level is shallow. Periclinal wall is thick with striate texture; convex shaped,



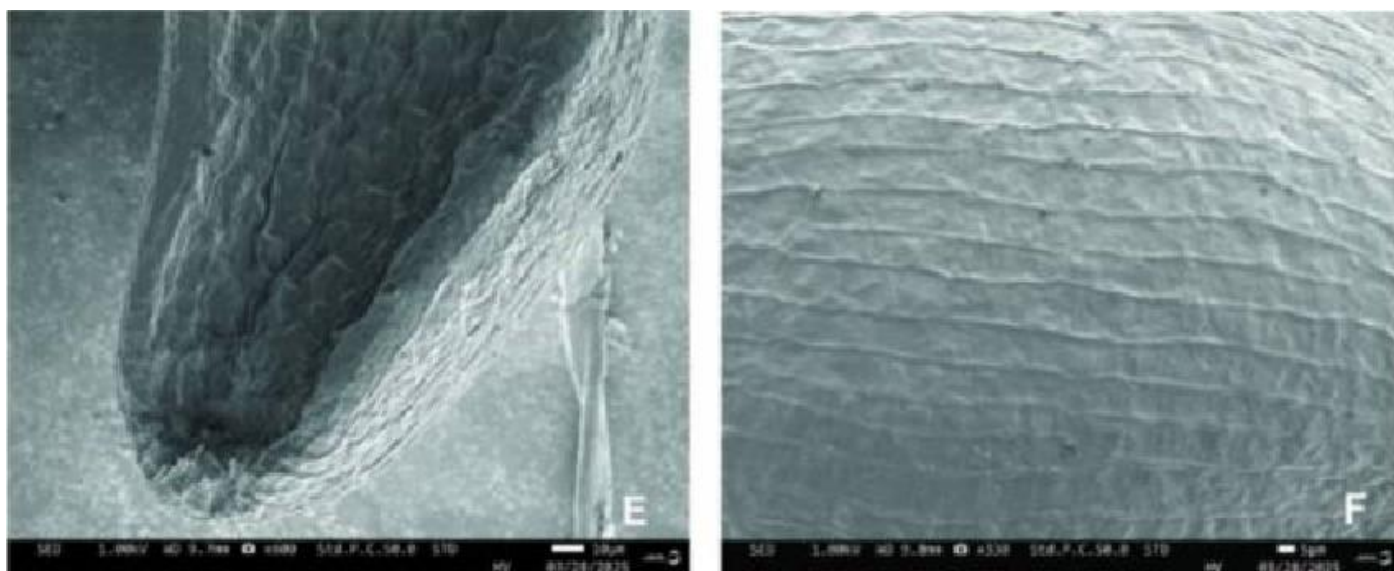


Fig. SEM micrographs of seeds of *R. occultiflora* (scale bar— fpm, 1011m & 50pm). A: antiraphal face; B&D: raphal face. C: length and width; E: hilum; F: Seed coat surface

DISCUSSION

In this study, we found that, in most species, the seed coat is reticulate type. *R. indica* and *R. cheruchakkiensis* shows the presence of trichomes on seed coat surface. In the present study, we also noticed that limited number of seeds, IO- 12, are seen in all the studied species and almost all are different sized. The species like *R. kasaragodensis*, *R. mexicana*, *R.*

meenkulamensis are highly endemic and distributed in specific localities of lateritic area, Seeds of *R. kasaragodensis* are spherical and 12 in number in each fruit- Placentation is axile in all studied species; seed coat surface is smooth in appearance.

EDAX ELEMENTAL PROFILE:

The EDAX (Energy Dispersive X-ray Analysis) elemental profiling of *Rotata* seeds elucidates a quantitative distribution of vital minerals and elements (Fig 15-24). The EDAX spectrum delineates significant elements, with pronounced peaks for carbon (C), oxygen (O), magnesium (Mg), potassium (K), alongside trace quantities of Sodium (Na), Aluminium (AL), Silicon, Sulfur (S) and Calcium (Ca), Carbon and Oxygen acts as vital regulators of seed dormancy, longevity and seed germination. Calcium and Potassium gives structural stability and initial nutrition to seed coat. Potassium is present at intermediate levels and is recognized for its role in the regulation of turgor pressure in seed coat. Micronutrients, including Silicon and Aluminium, although present in diminished concentrations, are essential for providing structural rigidity and cellular health. Chlorine controls chemical scarification and also releases dormancy.

These observations, represented in spectra from four distinct regions (Area 1, Area 2, Area 3, and Area 4, etc.), accentuate the variability in elemental distribution, thereby providing insights into the functional adaptations and mineral storage capacities of the seed. The EDAX spectra furnish a spatial analysis of mineral composition, illustrating how elemental concentrations fluctuate across the seed surface, which may correlate with specific physiological or ecological functions of *Rotata* species. The collective findings of these analyses demonstrate that *Rotata* species possesses both structural and biochemical adaptations that are highly conducive to its indigenous habitat. The microscopic examinations and FESEM (Field Emission Scanning Electron Microscopy) investigations of trichomes, seed coat configurations, and hilum shape in conjunction with the EDAX elemental profiling, present a holistic perspective on the seed's functionality. The FESEM analysis further elucidated the distribution of essential elemental profiles across the seed surface, indicating pathways for crucial nutrient interactions.

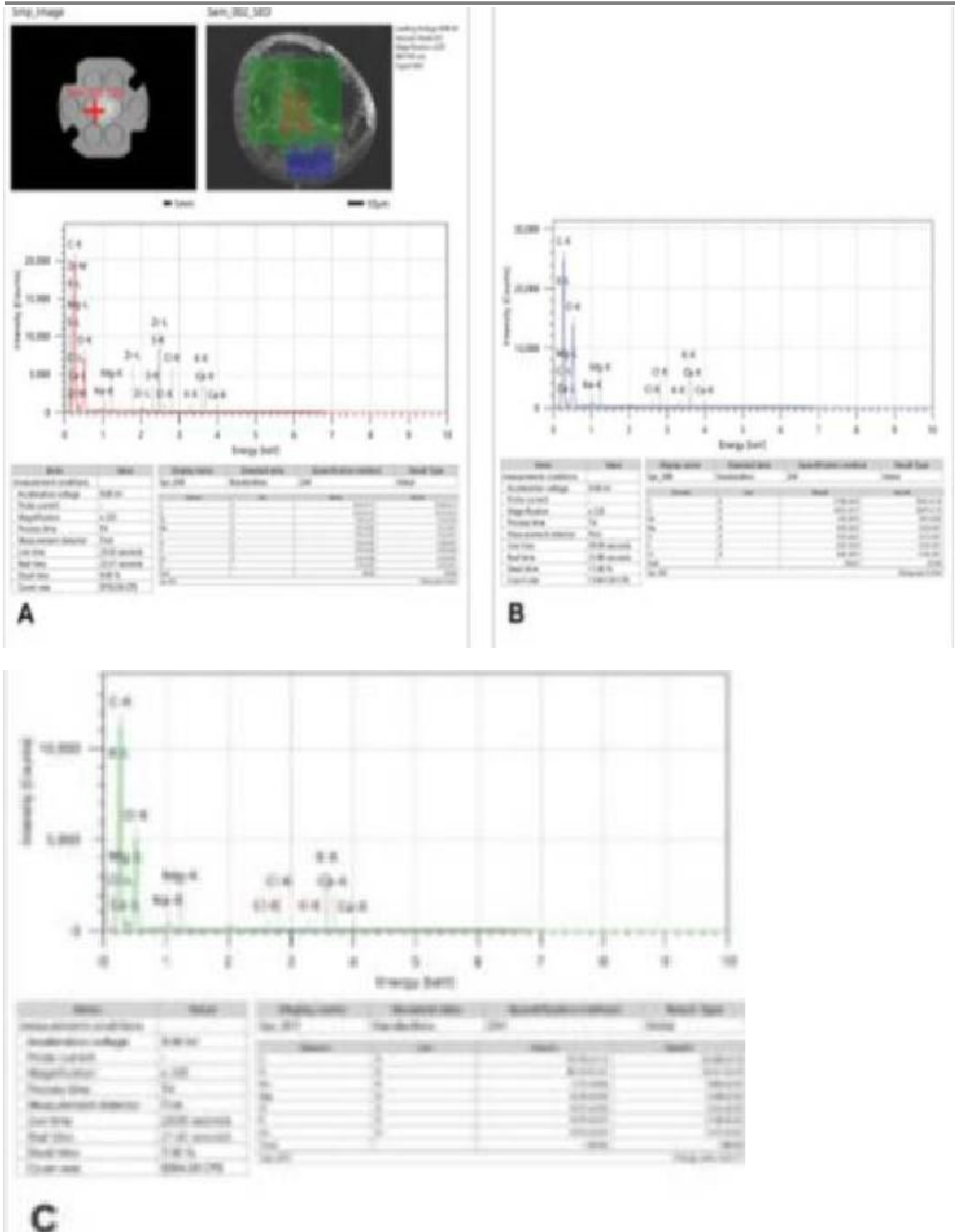
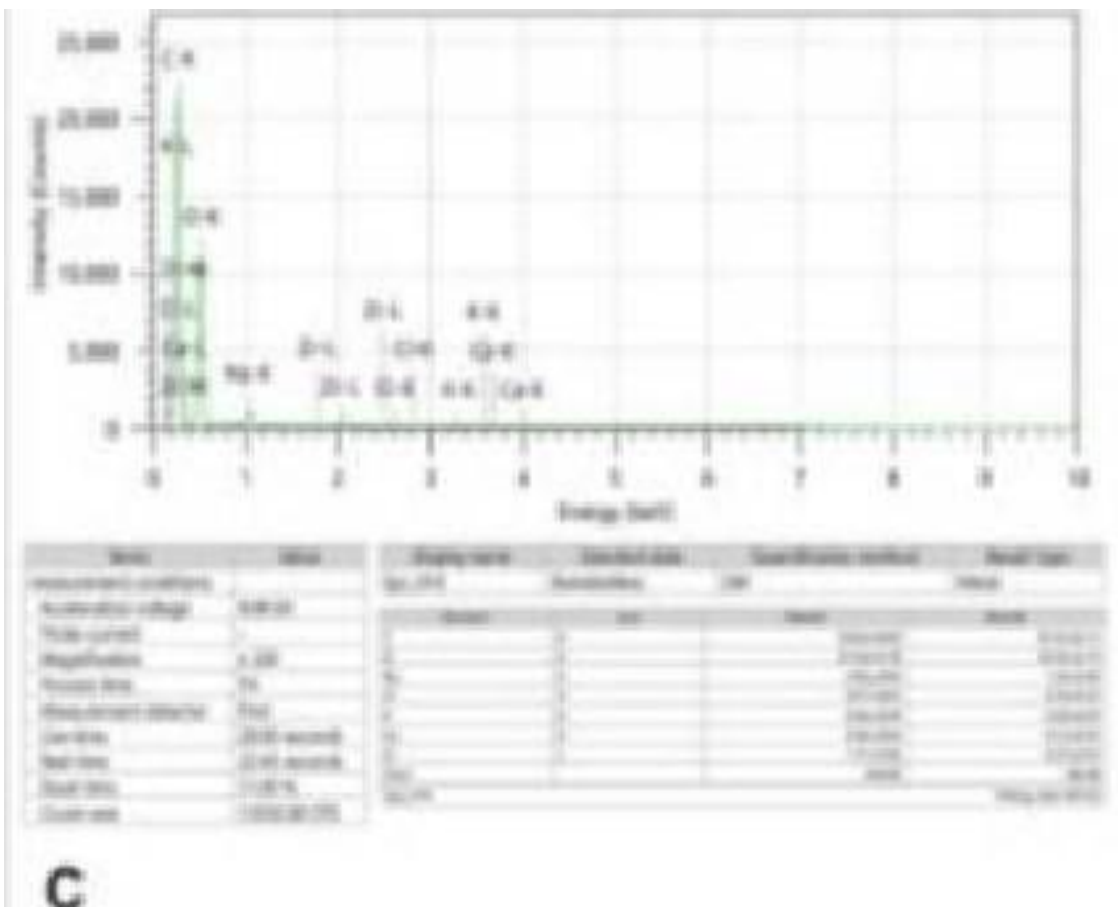
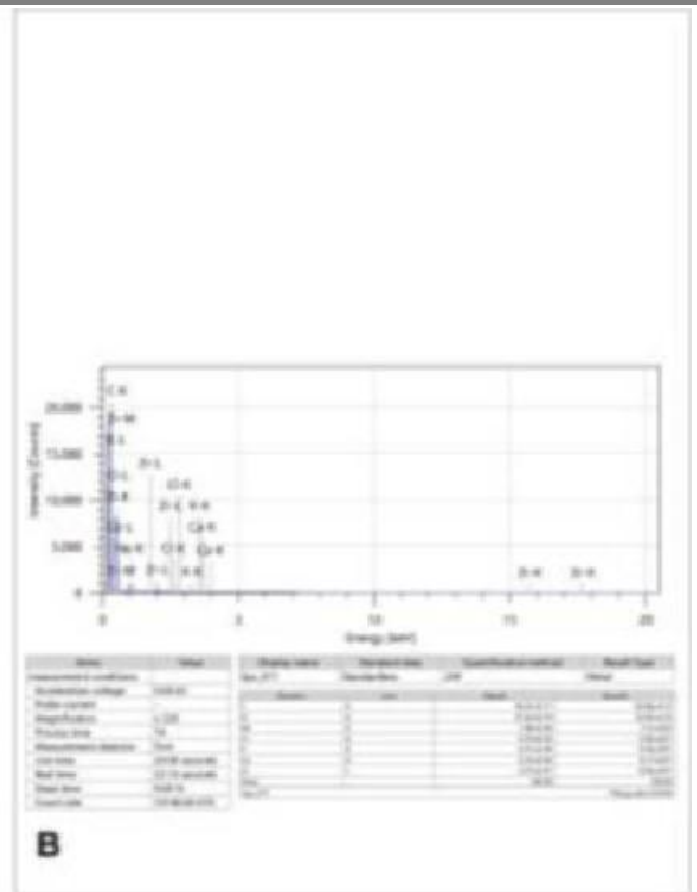
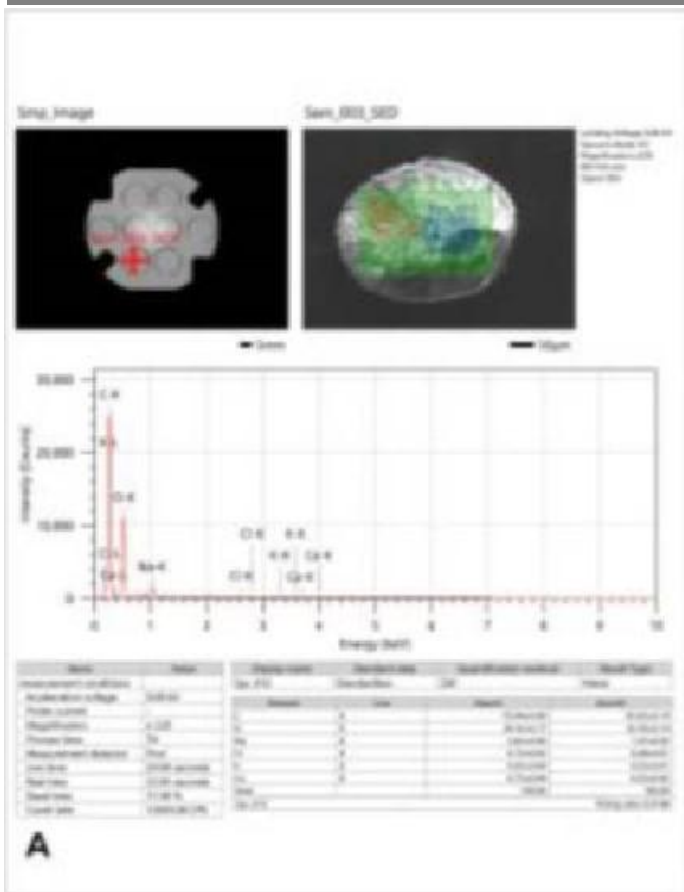


Fig. IS.profiling

or R *cheruchakkiensis* (scale bar=100µm)

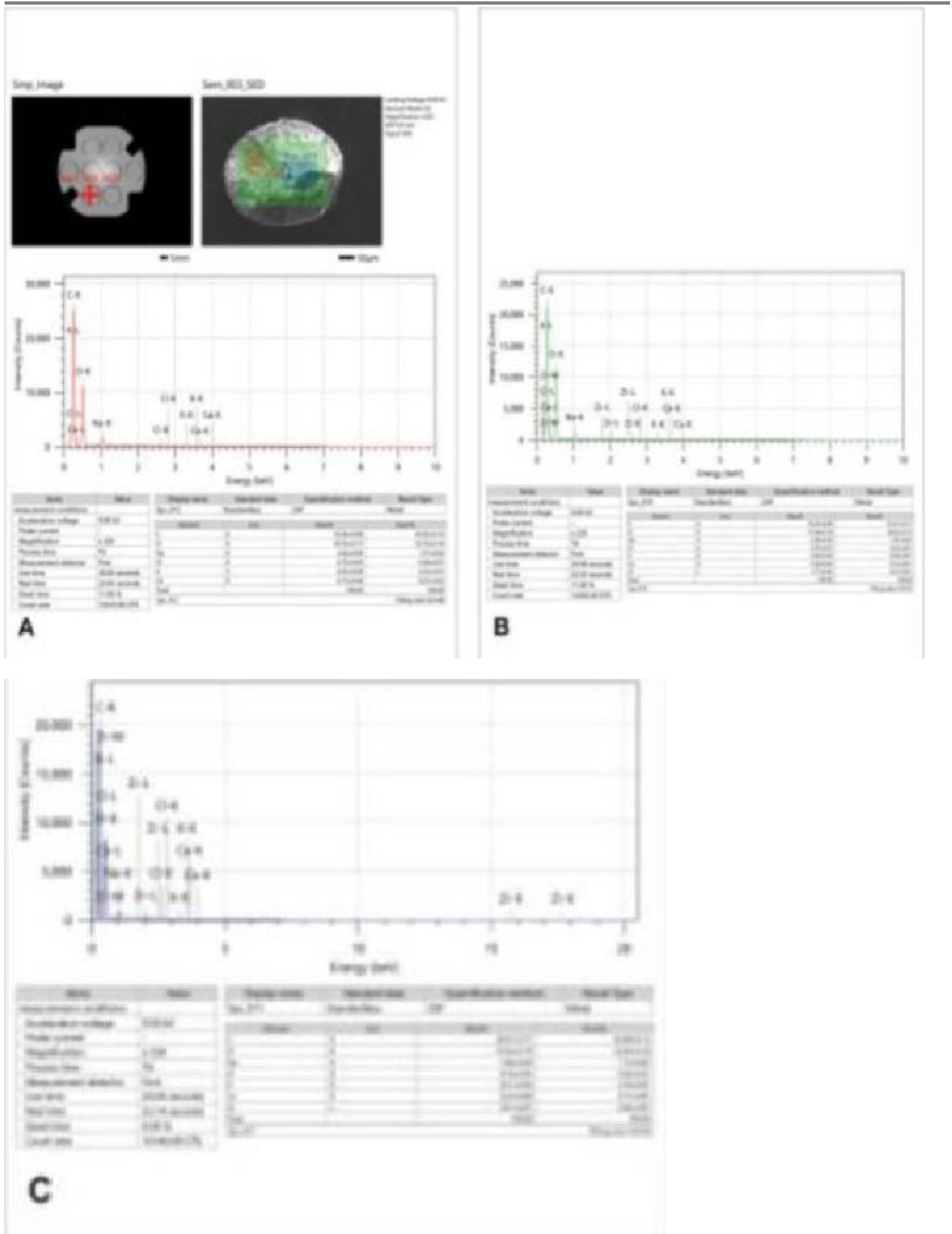
A: B: C: srs.013



Нд. 16. ЕОА\ проПНВК «4 Б.Г• А: Spc.009; B.

R. densiflora (scale
Spc-007

C:



17. [OAX pr•ПНВД @fseeas R *indica* (scale bar• юрт) A: Spe.012•, B. C: Spec.010

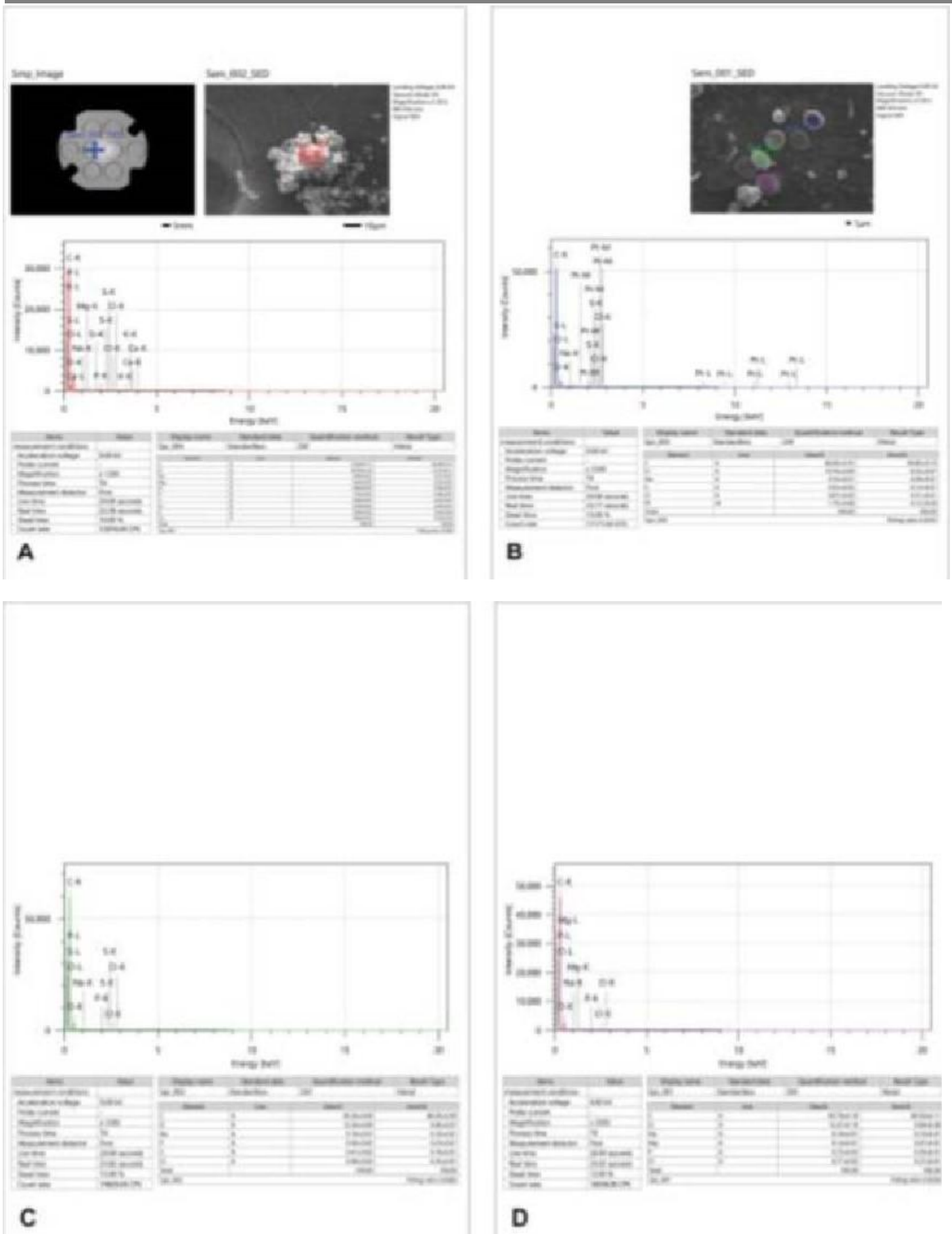


Fig. 18. E.OAN profiling of seeds or R bar—IO pm)

A: Spe.004;

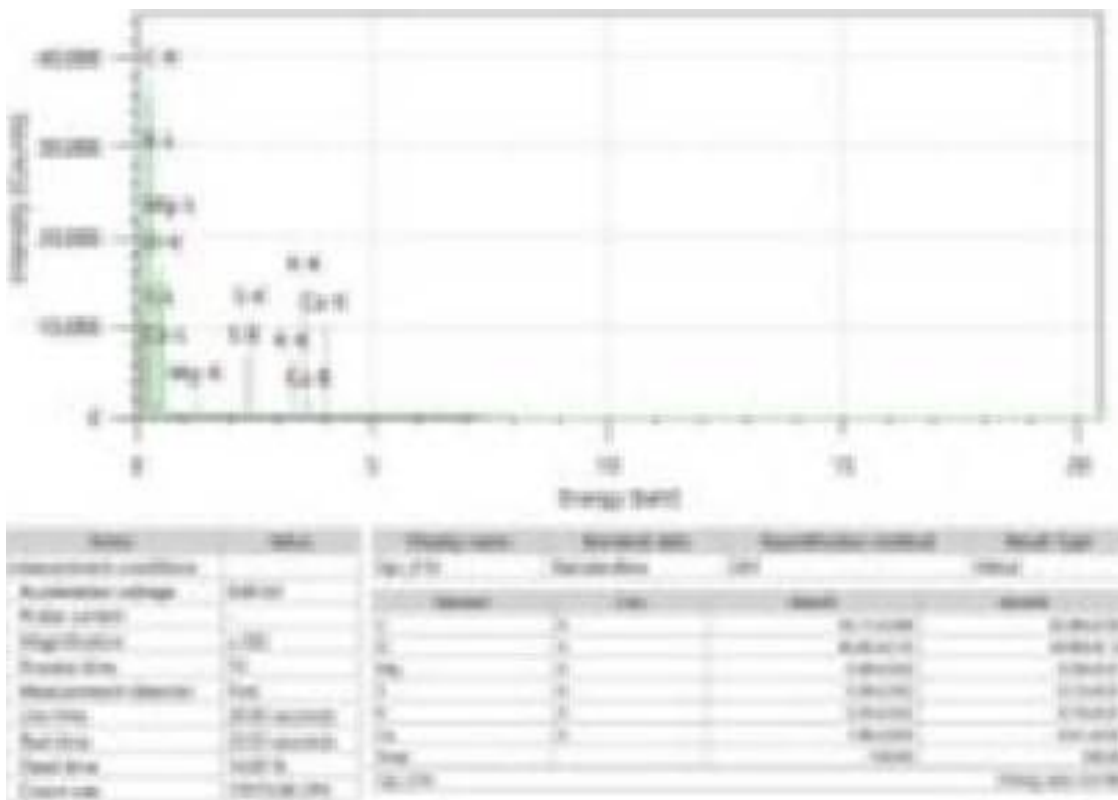
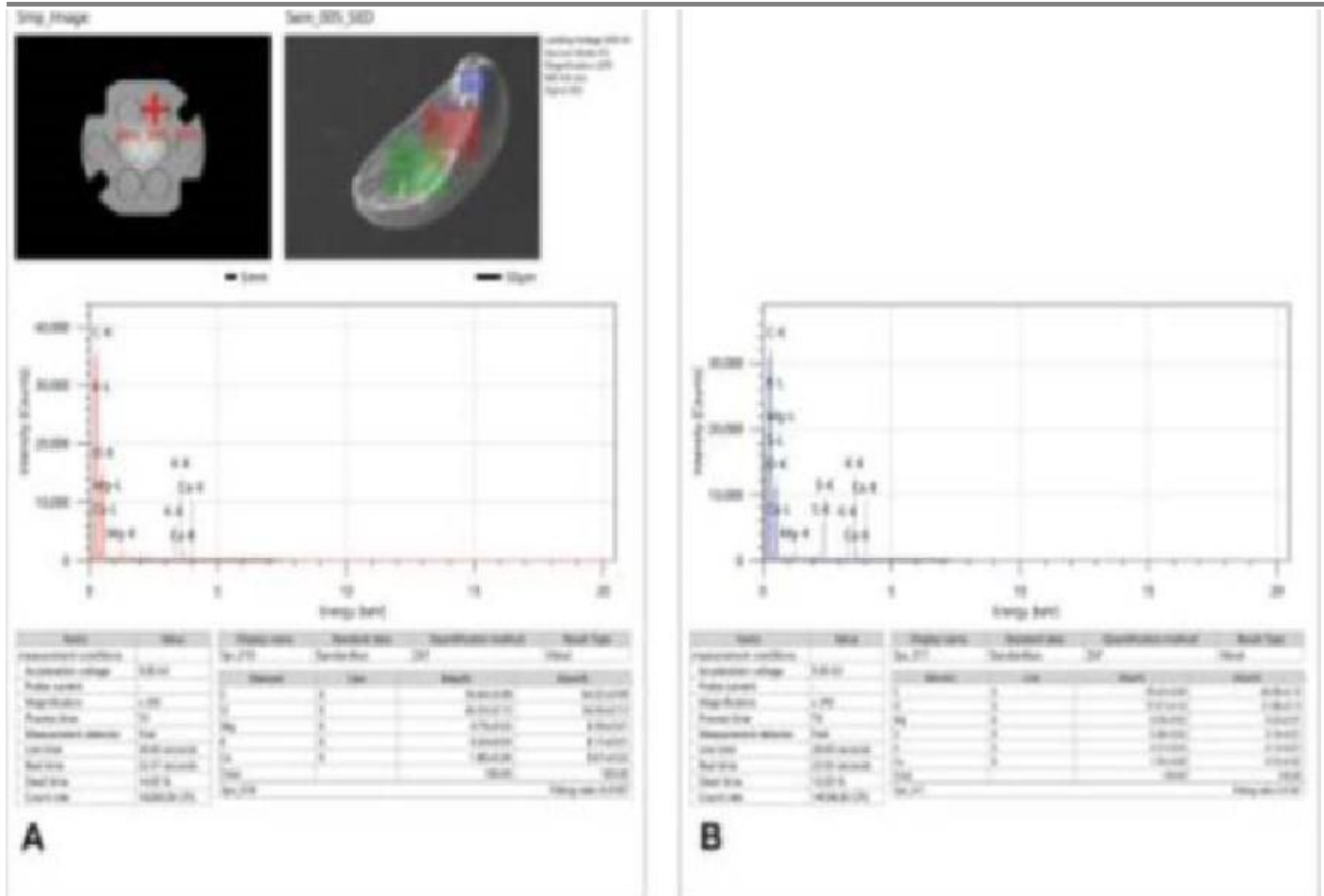
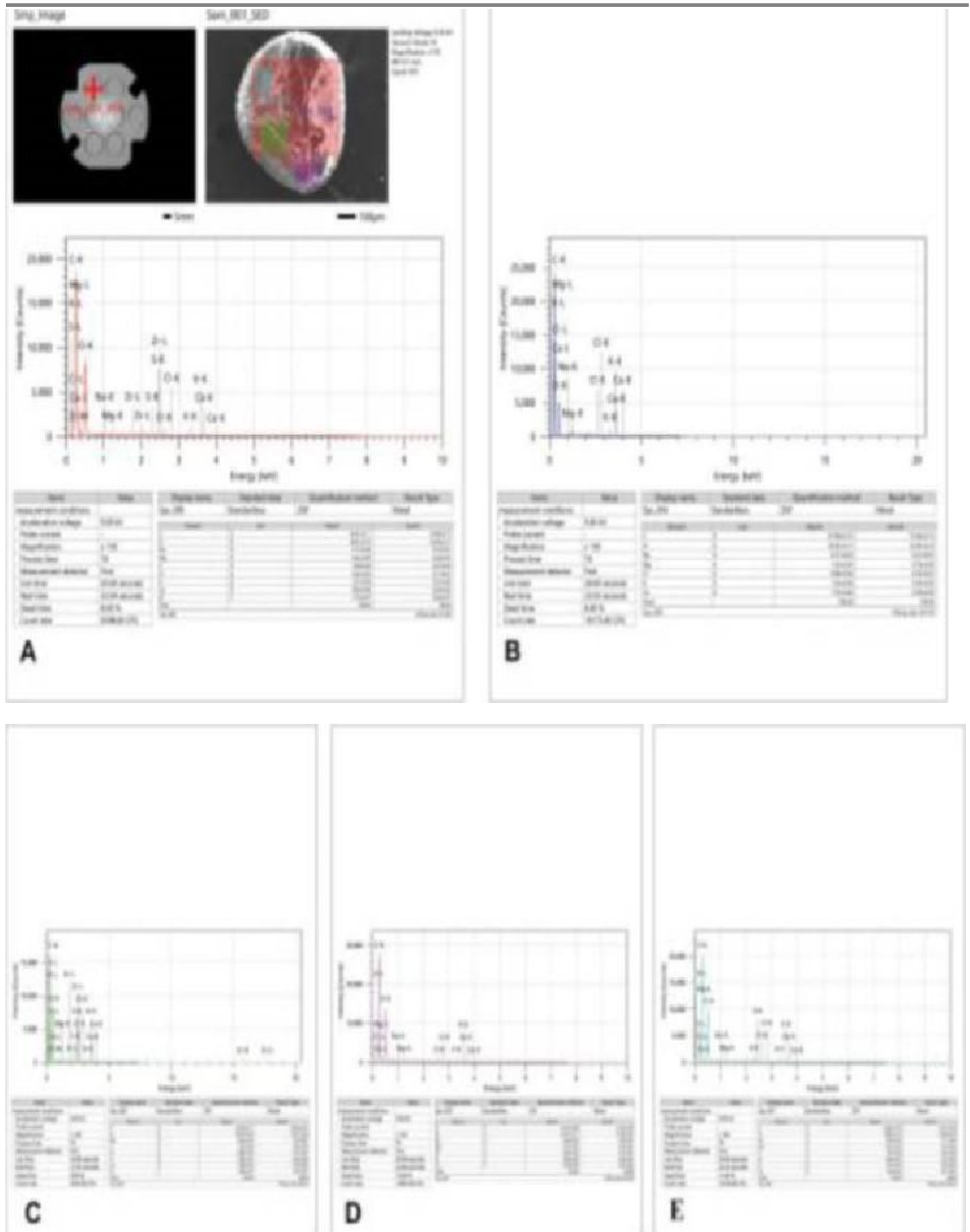


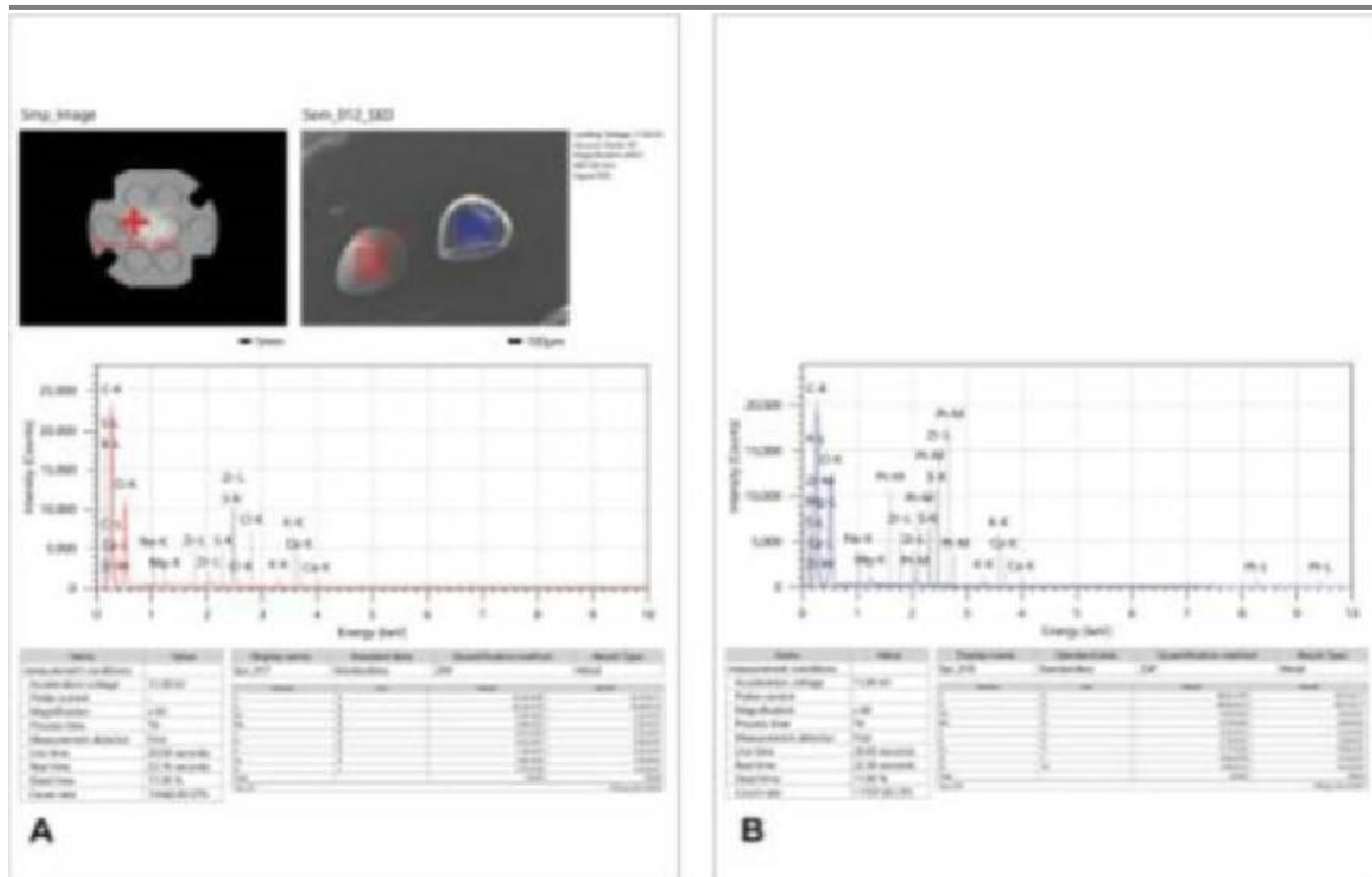
Fig. 19. GOA. \probing seed» от К. т.сг.пФ. Бар—50µm)



20. EDAX profiling of seeds of *R. malabarica* (scale bar=100 μm)

A: Spc-006; B: Spc-004; C: Spc-003; D: Spc-002; E: 001

(scale bar—100 μm)



EDAX profiling of seeds

Fig. 21. A: SPC-017; B: SPC-018 of *R. malampuzhensis* (scale bar=100µm)

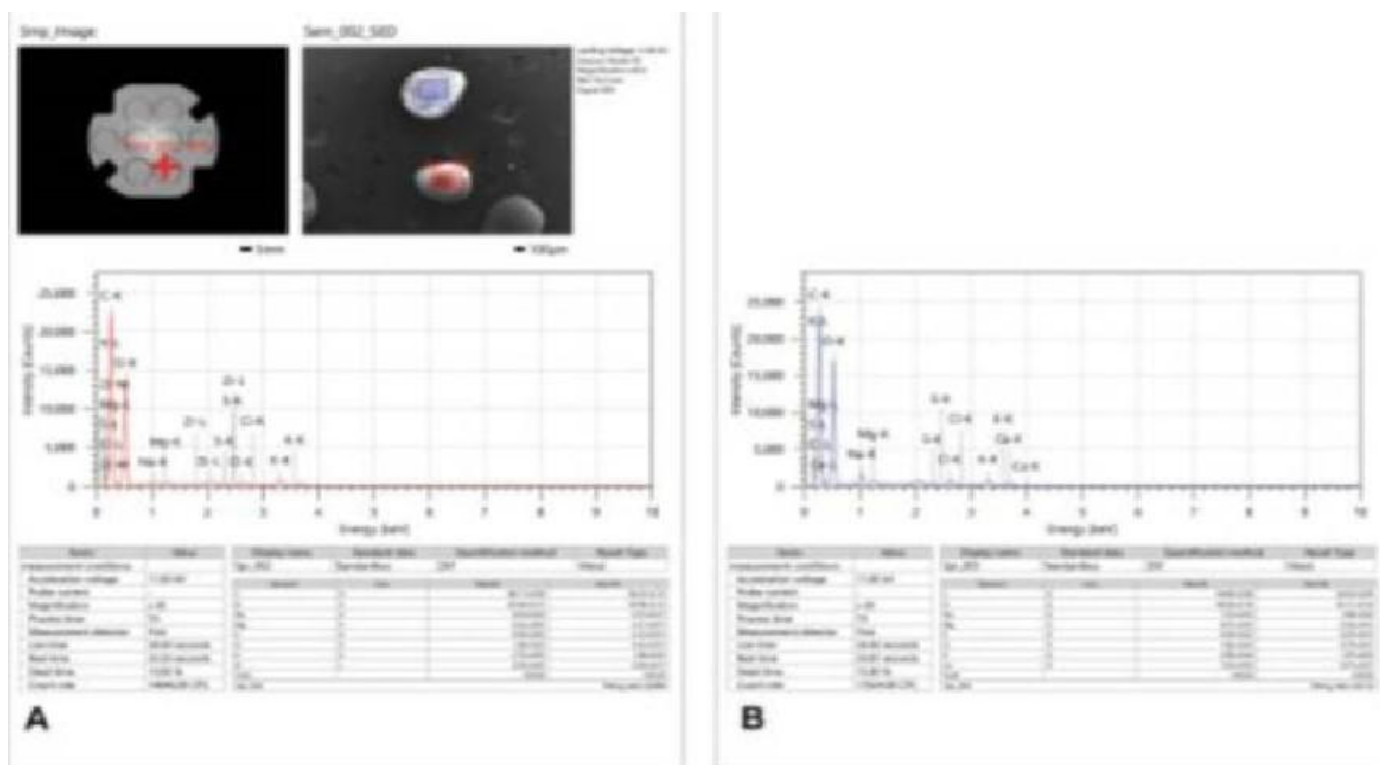


fig. [O AX] profiling of seeds of *R. meenkulamensis* (scale bar=100µm)

A: SPC4jO, B: Яхдюз

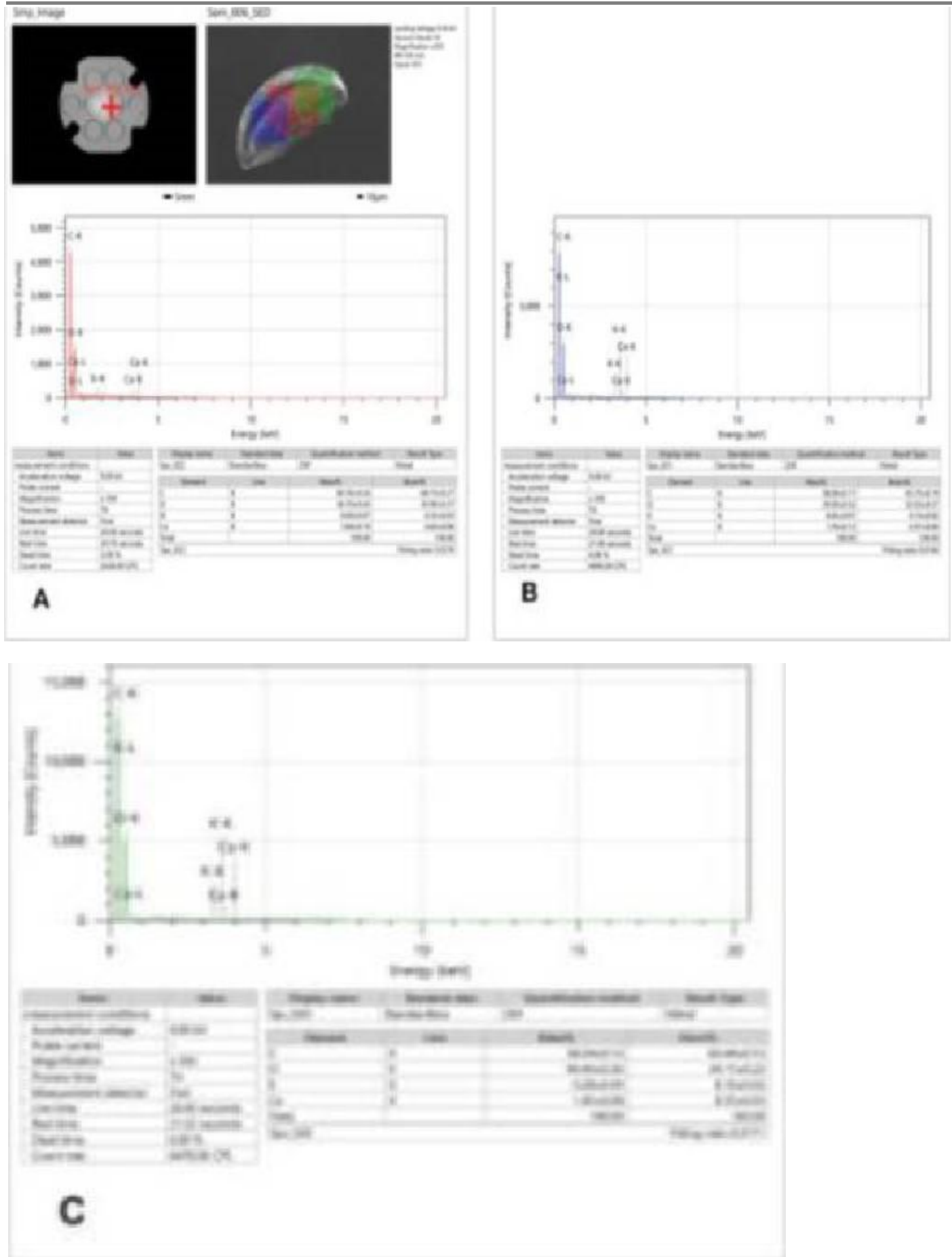
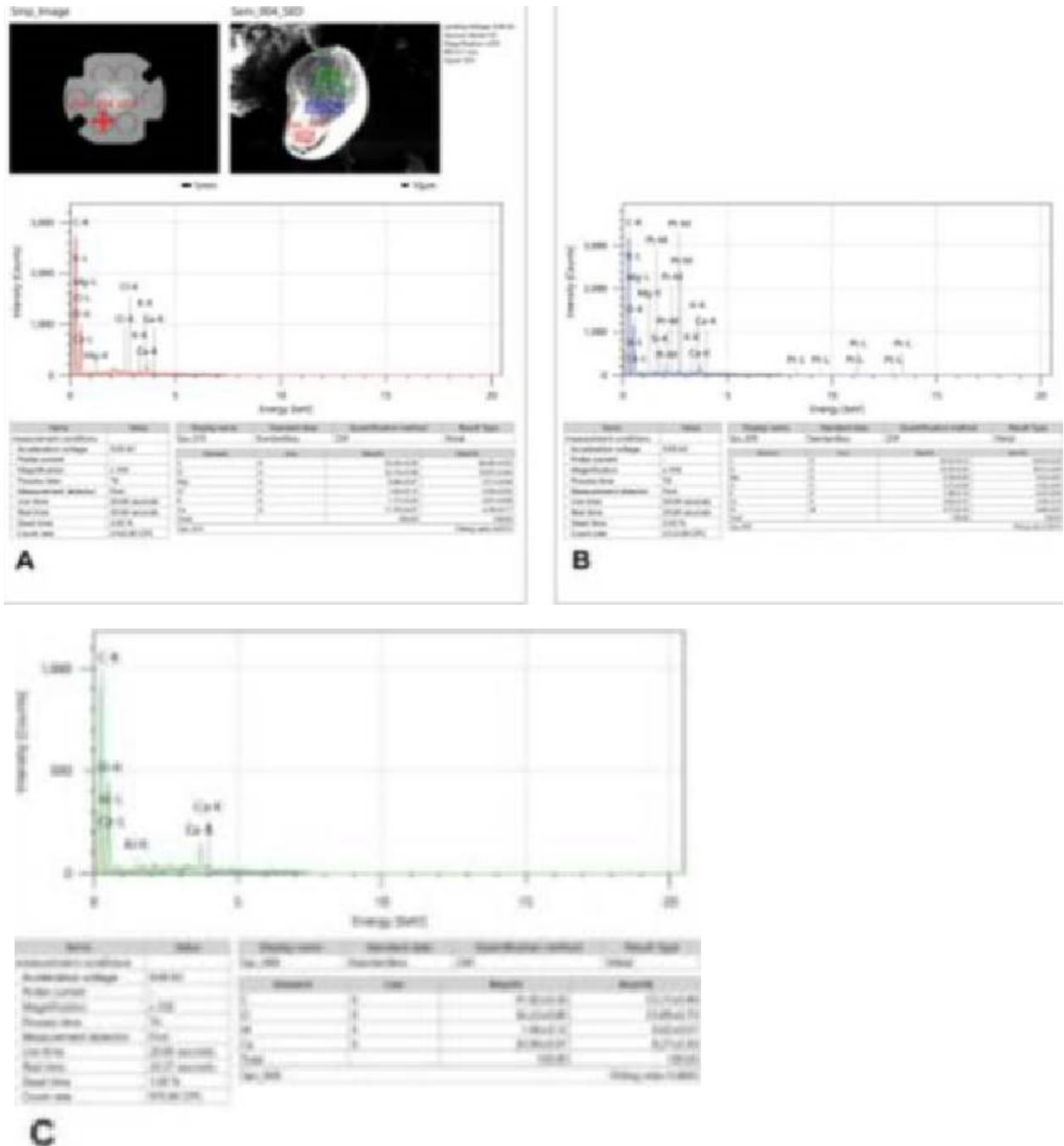


Fig.E DAX profiling of seeds Of *R. mexicana* (scale bar=10µm)

A: spc.022; B:

C: S,x.O-'0



profiling of seeds of *R. occultiflora* (scale bar=10µm)
010; B: Spc-009; C: Spc-008

fig. 24.[OAX |

CONCLUSION

From the above results, it can be seen that a clear eut distinction can be made between taxa based on the main external morphology of seeds. Seed morphological characters provide an aid in distinguishing various species and support their placement in different clades, The present study indicates that the use of seed morphological characters is a useful parameter for species identification of species in the genus *Rota/a*. In addition to the morphological characters of seeds, texture and sculpturing of features of the external seed coat show variations

among species and can be proved essential systematically, It can be concluded from the findings of the current studies that macro and micromorphological characteristics of seeds are considered diagnostic at both generic and specific level of plant species among the wide range of taxa. We recommend that future studies on Rotata plants with special reference to macro and micromorphological characters of seeds will provide a new perspective for understanding the habitat and dispersal features of the species, Moreover, the elemental composition of seeds of Rotata is significantly impacted by environmental variables, including the quality of water and soil, Seed coat act as a source of various essential and nonessential elements, which may shows slight variation in quantity according to the plant species. Lateritic soil is acting as source of various elements. It may also affect the plant as it is usually growing in lateritic pools and marshy areas.

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